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# Terminalia in the lower Eocene of southeastern North America

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(WITH FIGURES 1–6)

During the field studies of E. W. Hilgard, which culminated in his classic volume on the Geology and Agriculture of the State of Mississippi, published in 1860, he encountered fossil plants at several localities. These he collected and submitted to Leo Lesquereux, the nestor of American paleobotanists. The latter's botanical determinations of these fossil plants are listed in connection with Hilgard's account of the stratigraphy of Mississippi.

Among the lists of fossil plants occurs a species of *Terminalia* (*p. 113*), obviously based upon pictures of leaves which European students of Tertiary floras had referred to that genus, and not upon comparisons with the leaves of existing species of *Terminalia*. The leaf from Mississippi so-named by Lesquereux, of large size and characteristic in form and venation, has since been found to have a considerable geologic and geographic range.

When Lesquereux came to publish an illustrated systematic account of Hilgard's collections, which he did in 1869, he had changed his opinion of the relationship of this particular species and transferred it to the genus *Magnolia*,[1] a member of a different and quite unrelated family, and thereafter he consistently referred all large and simple fossil leaves to the genus *Magnolia*, in which practise he has been followed by Newberry, Hollick, and Knowlton.

When I was preparing the account of the Wilcox flora which was published in 1916,[2] I compared these and other related

[1] Lesquereux, Leo. Trans. Am. Philos. Soc. **13**: 421. 1869.

[2] Berry, Edward W. The lower Eocene floras of southeastern North America. U. S. Geol. Survey Prof. Paper 91: 1–481. *pl. 1–117*. 1916.

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leaves with those of recent species of *Terminalia* and *Magnolia*, and reached the conclusion that, in all of their essential features, they resembled the former and differed from the latter genus. This conclusion, necessarily not beyond doubt when based upon the foliage alone, was corroborated by finding in association with the leaves described as *Terminalia Hilgardiana* (Lesquereux) Berry, nut-like fruits which were very similar to those of existing Terminalias that have dry wingless fruits like those of *Terminalia Catappa* L., and these were described under the name *Terminalia Wilcoxiana* Berry. These fruits and the leaves of *T. Hilgardiana* have been utilized in making a restoration of a fruiting branch, which will be published eventually in an elaborate account of the Wilcox flora now in course of preparation.

A somewhat less important argument for regarding these leaves as those of *Terminalia* in preference to *Magnolia* is furnished by the fact that nowhere among the tons of Wilcox plant material that has passed through my hands have I found any traces of seeds or fruits which could be referred to *Magnolia*. It is true that certain leaves from the Wilcox have been recorded as so-called species of *Magnolia*, but this seeming inconsistency resulted from identifications of forms in the Wilcox which had been described by others from outside the Atlantic Coastal Plain, and which were of considerable chronologic interest, and about whose botanical relationship I was at a loss.

Still another feature of some weight is furnished by the botanical facies of the Wilcox flora as a whole, and particularly the facies of the richest and best represented horizon in the Wilcox, namely toward the top of the Holly Springs sand. Here the dominant ecological group is a plant association, which might properly be termed beach jungle except for the fact that this term has been used by students of the recent flora almost entirely for such a grouping on tropical strands, and the Wilcox strand was not tropical. In this environment, which has been determined with considerable precision by inductive and not subjective logic, *Terminalia* would be much more likely to be represented than would *Magnolia*.

There is therefore every line of evidence leading away from *Magnolia* and toward recognizing *Terminalia*, and this identification has received additional confirmation during the past year by the discovery, at four different localities near the

top of the Holly Springs sand in western Kentucky and Tennessee, of a second and entirely distinctive type of *Terminalia* fruit. It is the purpose of the present note to name and describe this fruit, and to present a restoration based upon it and combined with the associated foliage known as *Terminalia Lesleyana* (Lesquereux) Berry. The new species based upon fruits may be described as follows:

**Terminalia vera** Berry, n. sp. (FIGS. 1–5)

Fruits elongate, bi-alate, elliptical in outline, pedunculate; consisting of an axial, thickened seed cavity, fusiform in shape, ligneous in appearance as preserved as a carbonaceous impression. This seed cavity extends from near the base, almost to the tip of the fruit, and occupies from ⅓ to ½ of its transverse diameter. The wings are scarious, very finely veined, truncated or rounded proximad, and more or less emarginate distad, with entire but slightly irregular margins. The venation is thin and largely immersed in the wing substance, which is of considerable consistency; it is reticulate and approximately of one caliber throughout; over the seed cavity it is prevailingly of longitudinally elongated meshes, the long axis of the meshes curving outward toward the margins of the seed cavity where they pass to the wings. On the lower part of the wings, and correspondingly near the base of the seed cavity, the long axis of the meshes is transverse, and these become more ascending on the wings as the distance above their base increases.

These fruits vary considerably in size and in the degree of elongation of their elliptical outline. The peduncle is short, stout, generally curved, and about 4 mm. in length; it is usually broken off before fossilization, but is preserved in some specimens. The fruit itself varies from 1.7 to 3.5 cm. in length, and from 0.9 to 1.5 cm. in maximum width, which is midway between the apex and the base.

These fruits are readily distinguishable from the somewhat similar fruits found in the Wilcox deposits, which have been referred to the genera *Ptelea* and *Dodonaea;* and to the botanically unassigned species, *Carpolithus prangosoides* Berry, so that it is unnecessary to enumerate the differences in the present connection. They agree in all of their features with the fruits of the existing species of *Terminalia* which have bi-alate fruits, and there is not the slightest doubt but they represent a lower Eocene species of that genus. They have been discovered at the following localities in three adjacent counties: Grable pit and

Puryear, Henry County, Tennessee; Thompson & Barksdale prospect, 2 miles south of McKenzie, Carroll County, Tennessee; and 100 yards East of the Bell City Pottery pit, Calloway County, Kentucky.

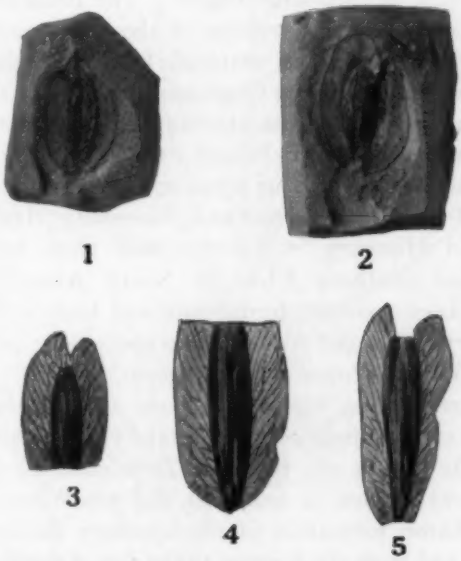
I have made comparisons with as many winged fruits of *Terminalia* as I could obtain access to, although I have seen only about ten per cent of all of the existing species. All of the American bi-alate forms that I have seen have the wings transversely extended, so that the fruits are much wider than they are high. On the other hand existing forms with the proportions of the fossil are to be found in both African species, as for example *Terminalia Brownei* Fresen. of Abyssinia; and Asiatic species, as for example, *Terminalia Darlingii* Merrill of the Philippine Islands. In fact the last is extremely like the fossil in every way. Whether the greater resemblance of this lower Eocene American species to existing African and Asiatic forms has any significance, or whether there are existing American species with fruits of this character I cannot say—I have not seen any.

The genus *Terminalia* comprises over 100 existing species, found in all tropical countries, and usually segregated into four sections according as the fruit is fleshy, nut-like and ligneous, or variously winged. The present fossil form obviously belongs in the section Diptera, which is represented in modern floras in South America, Asia, and Africa.

The family to which *Terminalia* belongs is usually termed the Combretaceae, although it is often called the Terminaliaceae. It includes about 16 genera and nearly 300 existing species of shrubs, trees, and tropical vines; with simple, entire, coriaceous, persistent, exstipulate, alternate or opposite leaves, which are often of large size. The inflorescence is racemose or capitate; and the flowers are regular, perfect or polygamous, often apetalous. The stamens are two or three times as numerous as the petals, and the one-celled ovary develops into a drupaceous or berry-like, often ligneous or winged, indehiscent fruit, which contains a single seed without endosperm, and is crowned with the accrescent calyx in many species.

The existing species are all tropical or sub-tropical, ranging from 34° north to 35° south latitude; and a relatively large number are littoral or strand types. The various continental areas contain the following number of peculiar species: America

75, Africa 85, Madagascar 36, Asia 57, Australia 23. About ten or a dozen species are found in more than one of these areas, and there is a remarkable number of identical or closely related species in tropical west Africa and tropical America, the genera *Cacoucia*, *Conocarpus*, and *Laguncularia* having identical species in both regions.




FIGS. 1–5. Fruits of TERMINALIA VERA Berry, n. sp. 1, from Grable pit, Tenn. 2, from 100 yards east of Bell City Pottery pit, Kentucky. 3–5, from Puryear, Tennessee.

The geological history of the family is exceedingly incomplete and will doubtless remain so until the geology of the equatorial regions is more thoroughly explored. At least the genera *Terminalia*, *Conocarpus*, *Combretum*, and *Laguncularia* have been found fossil, and all of these are represented in the Wilcox flora, where both leaves and fruit of *Terminalia*, and both leaves and flowers of *Combretum* have been discovered.

No genus of the family has been identified with certainty from rocks earlier than the Tertiary, although leaves referred to *Conocarpites* have been described from the Upper Cretaceous (Tuscaloosa formation) of Alabama, others referred to *Terminaliphyllum* from the Upper Cretaceous (Perucer beds) of

Bohemia, and still others have been referred to *Combretiphyllum* from the Upper Cretaceous of western Africa (Kamerun).

In addition to the two kinds of leaves and two kinds of fruits of *Terminalia* found in the Wilcox Eocene, the genus is represented by leaves of two species in the middle Eocene (Claiborne group), and one of the latter continues into the upper Eocene (Jackson group) in this same region. The foregoing represent the earliest authentic occurrences of the genus, although it is represented by undescribed material from the middle Eocene of Italy. During succeeding Oligocene time five or six species of *Terminalia* are recorded from the northern shores of the Mediterranean in southeastern France, the Tyrol, Italy, Styria, Carniola, and Greece. About seven species have been described from the Miocene in Switzerland, Germany, Italy, Croatia, Bohemia and Hungary in Europe; and from the Island of Trinidad, and southern Chile in South America. Pliocene species have been recorded from Spain and Italy in Europe; and the characteristic winged fruits of two species are present in the Pliocene of Bolivia (Potosi and Corocoro).

The accompanying restoration, one third natural size, is based upon a careful study of the habits of various existing species (FIG. 6). The leaves are those of *Terminalia Lesleyana* (Lesquereux) Berry, which is found in the pre-Wilcox Eocene of Texas, the Raton formation of the southern Rocky Mountain Front range, and from the bottom to the top of the Wilcox group. With these leaves I have associated the bi-alate fruits described in the preceding paragraphs. The fruits and leaves have not been found in association at the same outcrop, but both occur at identical horizons in the Wilcox.

The only uncertainty about the restoration is the use of the leaves of the *Terminalia Lesleyana* type rather than those of the *Terminalia Hilgardiana* type. The latter are equally widespread in the Wilcox, but since they are associated with the nut-like fruits of *Terminalia Wilcoxiana*, I have assumed that the last two probably represented a single botanical species. In any event the two types of leaves are not very dissimilar, so that I believe that the restoration presents an essentially correct idea of the appearance of a lower Eocene form of this interesting genus, one that is far more objective than would be the mere portrayal of single detached leaves and fruits.

FIG. 6. Restoration of a lower Eocene species of TERMINALIA from the Mississippi embayment region, 1/3 natural size.

# Botanical problems of American tropical agriculture[1]

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In the field of the plant sciences there are nowhere so many unsolved problems as in the Tropics. There the frontier offers to the pioneer investigator a chance to open new roads to knowledge. There we find outstanding opportunities for service by science in the interest of progress in two hemispheres, that have become closely interdependent.

The people of tropical countries derive their principal livelihood from agriculture, and the products of their fields and forests form the greater part of their export trade. Their future development will continue along agricultural lines, and their expanding production of raw materials will be sent north to be worked up in our factories or to feed an increasing urban population. The United States, on the other hand, has become an industrial nation, dependent on the outside world for many essentials, and will need more and more to buy plant products from the Tropics, in exchange for manufactures.

Our tropical importations are, in fact, peculiarly indispensable threads in the interwoven fabric of our modern civilization. Consider rubber and other gums and resins; coffee, tea, and chocolate; sugar; oils; fruits and nuts; the fibers, sisal, jute, abaca; dyes; spices; and woods.

Our annual purchases of these important tropical commodities which can not be produced in the temperate zone exceed a billion and a half dollars. We get every year in round figures, tropical sugar to the value of 400 millions of dollars; coffee, 240 millions of dollars; rubber, 180 millions; tobacco, 70 millions; coconuts, tea, and cacao about 30 millions each; bananas, 20 millions; and many other things in proportion.

The first four items in the list of our chief imports from all countries are silk, cane sugar, coffee, and rubber. The sugar production of Cuba alone is approximately equal to the entire sugar consumption of the United States.

[1] Invitation paper read at the joint meeting of Section G (Botanical Sciences), American Association for the Advancement of Science, the Botanical Society of America, the American Phytopathological Society, and the American Society of Plant Physiologists at Kansas City, Mo., 29 December 1926.

Food supplies may be drawn from the Tropics to postpone to the distant future the time when food production will fail to keep pace with increasing population. The greatest contributions will be of sugar, oils, and fruits. Particularly large areas, now unused, are suitable for sugar cane, for coconuts and other oil palms, and for those most nutritious of fruits, the banana and the avocado.

The expanding culture of these and other food plants will bring up many new problems to the plant scientist for solution. The outstanding need in the Tropics today is for research. The extent to which they will be able to respond to calls for more food, fibres, rubber, etc., will depend upon the extent to which scientific methods are applied in their production. There are vast areas of unused land, in a climate where plants grow the year around, but at the present time, with some exceptions, agriculture is not highly developed. The principal crop plants are but little improved, and are grown, harvested, and handled by primitive methods; consequently the product of the unskilled labor of the tropical worker is barely sufficient to feed and clothe him and does not allow much of a surplus for export. We can depend on medical science to make the Tropics healthful, and on inventors and engineers to introduce in due time labor-saving machinery and effective means of transportation of the products to market by road, by rail, and by water, but the plant research needed is not yet started in any adequate manner.

Consider the vastness of the field. The area of tropical America is two and one-third times the area of the United States. It presents the most diverse conditions of climate, soil, and vegetation, so that the proper development of agriculture requires a large number of special investigations to cover the numerous crops in their local environments. The obvious problems needing scientific research are more numerous than in the United States; yet while we have over 300 stations for agricultural experimentation, there are in tropical America very few corresponding institutions, equipment, personnel, and financial support considered in the comparison.

This lack will not be met promptly by governments, and hope for support of research rests in large part on associations of producers or distributors of tropical products. Support of research by industrial corporations has become common in this

country, and we live in an age of marvels due to such research in chemistry, physics, and engineering, but it has not yet become so well understood that research in crop production is fundamental to continued prosperity.

The botanical problems awaiting solution cover the entire range of plant science. It is interesting to note that the principal tropical crops entering international commerce have been introduced from some other part of the world into the region where they are now grown. Sugar cane, which now dominates the West Indies, came from the Orient; rubber, native of the Amazon region, is grown principally in the far East; coffee, an African plant, is supplied to the world from Brazil; while cacao has made the reverse trip from South America to Africa. The Dutch East Indies are now growing the South American cinchona and the African oil palm. The banana and coconut, which we receive from the Caribbean regions, had their origin in the Pacific. We must conclude that the possibilities of plant introduction and acclimatization are by no means exhausted.

The improvement of tropical plants by modern methods of selection and breeding has barely begun. Such important crops as rubber, coffee, and cacao, and most of the tropical fruits, are still propagated by seeds. The perfection of methods of vegetative propagation and the replacement of existing cultures by special or named varieties would appear to be in the line of progress, and this opens up an interesting field of research in plant physiology on methods of propagation and the use of adapted stocks.

Plant diseases and insect pests are limiting factors in crop production in the Tropics. The culture of bananas has become impossible in places on account of the Panama disease. Budrot of coconuts has killed thousands of trees. The cacao industry of Ecuador seems to be threatened with disaster, owing to the spread of the witches'-broom disease. Sugar cane mosaic has cut into the profits of cane growers in many countries. The South American leaf disease of rubber may handicap the establishment of a plantation rubber industry in the home of *Hevea*. The destruction of the coffee industry of Ceylon and Java by leaf rust shows what may happen if this fungus should be brought to South America. In all these cases scientific investigations will require years of time, and should be begun before the critical need arrives.

Sugar cane problems are present in every country. In this period of intense world competition, sugar has become the cheapest food, and production of sugar cane at a profit depends on methods that will lower the cost of cane per ton. Cheap production in the Tropics has hitherto been based on the utilization of virgin lands, where cane grows almost without cultivation and without need for replanting for many years, but soon there will be no more forests to clear, and cane growing must pass from a pioneer condition of soil mining to a settled permanent agriculture. The plant specialist must reduce the hazard of crop losses by plant diseases and introduce new cane varieties locally adapted, disease resistant, with high tonnage yield, and richer in sugar.

Problems of maintenance of soil fertility in the older regions are already in evidence and will become pressing before research has provided solutions for questions of cover crops, green manures, legume inoculation, soil biology, and the fertilizer requirements of various soils and crops.

To a greater extent than at home there is need in the Tropics for breaking new ground in botanical research, to study methods of curing, fermentation, and extraction, and to search for useful by-products and new sources of oils, gums, latex, dyes, spices and fibres. Such investigations call for organized attack, for teamwork among botanists and with other branches of science. The plans should be well rounded, liberally supported, and carried on for many years. It may be predicted that there will be developed special crop stations maintained by the organized industries to supplement the work of governmental experiment stations, as exemplified by the Cuban Sugar Station of the Tropical Plant Research Foundation.

There is need in the case of each of the great tropical crop industries of a special survey to study the present conditions, to take an inventory of crop resources, point out and define the problems, diagnose the ills, and to outline a program of research, with recommendations as to equipment and personnel. Such a survey is now being undertaken by the Tropical Plant Research Foundation in the West Coast of Peru, in the interest of the sugar cane and cotton producers there.

*Rubber research needed.* We find ourselves now in a period of renewed interest in rubber production, when high prices have

led to a re-survey of the tropical world to find the most favorable location for new plantations. If rubber culture is to be successfully extended, much botanical research should be begun at once and liberally supported by the industry, for while much has been done on rubber, we have much yet to learn about the rubber tree and its requirements as to soil, location, and culture, the production of latex and its extraction, systems of tapping, bark renewal, and diseases associated with tapping. Most of the planted rubber trees are seedlings, and of course all the wild ones, yet it is said that there is a variation of 1000 per cent in the productivity of different trees. Consequently there is a field for the physiologist to investigate means by which the profitable trees may be selected and propagated vegetatively.

Rubber is subject to some serious diseases. A leaf disease in its native home has interfered with plantation enterprises, but Rands thinks it possible to select resistant types, and such attempts should not be delayed until the disease spreads to other centers of production. Immediate steps should be taken to select out these disease-resistant trees and propagate from them. If a new rubber industry is to be built up, it will be economy to spend money at the start to protect it from destructive future disease attacks. Every such crop should have its special experiment station, and before new areas are opened up for planting, the investors should arrange for preliminary investigations of an ecological nature. These should include studies of soils and climate, the character of the natural vegetation as indicators of soil and climatic conditions, and of diseases and pests likely to attack rubber trees.

*Botany and dietetics.* Another group of plant problems has been brought forward by medical workers, who tell us that an adequate supply of fresh, palatable, and vitamin-rich vegetable food is essential to the health of the people of tropical America. We think of these lands as producers of food in variety and profusion, yet the traveler notices at once the general lack of fresh vegetables, and is prepared to believe that great numbers of people live on the verge of deficiency diseases, with reduced vigor and disease resistance.

This is more than a gardener's problem. It is a field for botanical research. What are vitamins? How do they trace back to the effect of light on chlorophyll? In what way are

these mysterious activators of our metabolism related to plant growth? Under what conditions are they formed? What is the value from a vitamin standpoint of the various food plants and their products? This is known for very few tropical fruits and vegetables. We also lack sufficient information on the chemical composition of tropical foodstuffs to properly estimate their place in the dietary.

Production problems are also numerous. They include plant introduction, to enable us to profit from the experience of the older civilizations of China, India, and other countries; control of pests; seed supply; plant breeding and cultural studies to improve quality and to overcome the handicaps of rainy seasons and dry seasons.

It has been proposed that such a series of studies be undertaken in coöperation with medical workers, and it is urged that this is the next and most important move for public health in the Tropics.

*The botany of forestry.* There is much botanical work needed to lay a foundation for the agriculture and forestry of the future. At the present time most of the undeveloped areas of tropical America are covered by forests. The native farmers clear areas of a few acres by cutting down the trees and burning them. After growing a few crops they are likely to abandon the clearing and make a new one, thus gradually nibbling away the forest. But when North American or other foreign interests enter the field to grow sugar cane or bananas, they follow the same system of slashing down the native vegetation, but on tens of thousands of acres, salvaging only a portion of the more valuable woods and often buying their building material in the United States. So much of the Cuban forests have thus been converted into cane fields that that country can not meet its own timber requirements until a program of reforestation and forest management is adopted and carried out.

That tropical forests are burned, when the world needs all its wood, results primarily from lack of knowledge of tropical woods and their uses, which exposes the Tropics to an uneconomic competition from the highly organized lumber industry of the United States, which can export building material cheaper than the local tropical supply can be brought in. In the United States there are great mills with railroads and every mechanical or

engineering facility, operating in pure stands. In the Tropics are mixed forests of strange species, without roads, tools, or mills. Obviously it is easier and cheaper to import lumber into the Tropics.

But we have reached the time when the forests of the United States are inadequate to meet our own needs. Of the hardwoods needed for cabinet work, tool handles, and numerous industrial purposes, we have no longer enough, and even if we begin at once to replant hardwood forests in the United States, there will be a long gap between the exhaustion of the present supply and the availability of a new crop. Consequently for many years we shall have to supplement our shortage by importing tropical hardwoods or by wood substitutes. There is an abundant supply of splendid hardwoods in the Tropics, but it is not an easy matter for a lumberman to enter a tropical forest, with its mixture of species, many of which are unknown to the trade and are shunned because their virtues are not understood, and organize a modern logging operation.

Several years of botanical research are needed in each region that is to be developed. The first need is for naming the trees. Much taxonomic work has been done, but not enough. Let us express the hope that Latin names once given, and especially the old familiar names, will be allowed to stand. Foresters and others use Latin names for convenience of reference, and are impatient and seriously handicapped when required to learn a new set of names.

The investigators will be asked to discriminate closely between species that resemble each other and are often marketed under the same name, and also between varieties that differ in some quality of wood. They will be called upon also to discover means of identifying trees without fruit or flowers, and logs without foliage, by character of bark, structure, color, or other properties. In addition to naming the species in the forest, it is needful to know how many of each kind there are in a unit area, and for each important species its relation to soil, elevation, rainfall, and other ecological factors. The records taken should include dates of flowering and fruiting of all important tree species, including all occurring in quantity, even if of no present market value. Common names should be recorded, and local lore concerning the value or uses of the woods or plants.

The collections should include absolutely authentic wood specimens as well as ordinary herbarium material, and there should be duplicates enough to deposit sets of woods in the National Museum, the Yale Forest School, the U. S. Forest Products Laboratory, and the Field Museum, and herbarium material at least to the National Herbarium, the New York Botanical Garden, and the Gray Herbarium, with some for foreign exchanges. Without fail, sets of both botanical specimens and woods should be deposited in the proper institution in the country where collected.

The plant pathologist and the entomologist should be brought into the forest to survey for diseases and insects that are injuring the wood or preventing the reproduction of the species.

The next move will be to procure logs of species positively identified, for wood tests by the Forest Products Laboratory, to determine strength, stiffness, hardness, finish, and numerous other qualities essential to industrial use. The woods may then be introduced to manufacturers for practical factory tests, and steps taken to secure the granting of lumber concessions, erect permanent mills, build roads, and conduct logging operations on a basis of 15 to 20 or more year rotations.

The plant scientist has another service to render in the interest of complete utilization of the resources of these mixed tropical forests; that is to identify and work out methods for utilizing other forest products and by-products, such as oils, resins, waxes, gums; or latex products like rubber, balata, and chicle; tannins; nuts; medicinal plants like quinine; and pulp wood. There is need also for the physiological chemist to perfect methods of extraction or utilization of these products.

Some of these tropical forests will be cleared for agriculture. The remainder should be placed under forest management, and this, together with the reforesting of already devastated areas, introduces problems for botanical research additional to those of forest utilization just mentioned. These lie mainly in the field of ecology and plant physiology, and relate to problems of successions of vegetation after clearing, the effect of fires, studies of reproduction of each important species, their optimum requirements of rainfall, temperature, soil, and elevation, their reaction to shade, their production of seeds, the care of seedbeds, and young plantations, reproduction by cuttings, and similar

problems. Scarcely any of this fundamental knowledge is available for the tree species of tropical America.

These various botanical undertakings should be correlated with the work of geographers, climatologists, and soil scientists already in progress in tropical America. There would result in the end a vegetation map and report on natural resources of the American Tropics that would be of fundamental value as a basis for the development of this important part of the world.

It is not too large an order to be taken on by our botanical institutions working in coöperation. Much has been done that can be utilized. Much is in progress; for example, the notable study of the flora of the Caribbean countries that has been carried on for years by Dr. Britton and his associates and the botanists of Washington and Harvard. Neither is the problem of financial support insurmountable. I have sought to point out that our botanical research may have value as a business investment for organized industry.

There are various difficulties in the way. It is said to be hard to find trained specialists, but I have no doubt that the law of supply and demand functions here as elsewhere, and that when taxonomists and ecologists come to have a real market value, these waning subjects will be revived. The language question is very important. With our present outlook, inability to read and speak Spanish is a serious handicap.

Let us encourage students to prepare for research work in taxonomy and ecology in order that they may take part in the development of tropical forestry and agriculture that is surely coming. Let us also do what we can to assist the Latin American universities to build up their courses in the natural sciences and give training in forestry and agriculture, and, to round out the program, establish a graduate school of tropical agriculture or an effective international relation between existing institutions to make it possible for our men to secure tropical experience, and for Latin American students to complete their education in the United States.

# The nuclear phenomena and life history of Urocystis Cepulae[1]

ALPHEUS W. BLIZZARD

(WITH ONE TEXT FIGURE AND PLATES 1–4)

Although there have been in recent years many papers on various problems dealing with reproduction in the Ustilagineae, there is still a divergence of opinion concerning certain fundamental features in their nuclear history, especially as to the origin of the nuclei that fuse in the young spore; the significance of conidial conjugation; the number of nuclei in the cells of the hyphae of the parasitic mycelium.

The literature relating to the smuts has been summarized adequately by Fischer von Waldheim (*21*)[2] Brefeld (*8*) and Lutman (*29*). I shall refer only to such studies as bear more directly on these problems. As is well known, Prévost (*37*) in 1807 was the first to observe the germination of the spores of the smuts. The Tulasnes (*45*) in 1847 seem to have been the first to observe the conjugation of the conidia. De Bary (*14*, *17*) Kühn (*27*) Fischer von Waldheim (*21*, *22*) Brefeld (*8*, *9*) and others have made studies of the life histories of the Ustilagineae and described a wide range of types of cell conjugation or anastomoses in the different genera and species of the group. Kühn (*27*) in 1857, in sections of the growing wheat sprouts, was the first to observe the infecting mycelial threads penetrating the host tissue. Kühn's observations were confirmed by Hoffman (*24*), de Bary (*16*), Wolff (*51*) Brefeld (*9*) and others. Brefeld's figures of the distribution of the smut hyphae in the tissue of the host in the case of *Ustilago Maydis* are perhaps the most adequate published so far for any smut.

The formation of the spores was studied by the Tulasnes (*45*, *46*) de Bary (*14*, *17*) Fischer von Waldheim (*21*) Wolff (*51*) Winter (*50*) Cornu (*10*) Woronin (*52*) and others.

De Bary (*15*) was the first to point out the analogy between the conjugation of the conidia of the smuts and the sexual fusions of the Conjugatae. Later (*17*, *p. 196*) he gave the following

[1] Contributions from the Department of Botany of Columbia University, no. 341.

[2] Reference is made by number (italic) to "Literature cited," p. 112.

evidence to show why the conjugation of the conidia should be considered as having sexual significance:

> First, the almost invariable occurrence of pairing under the normal conditions of germination; . . . Secondly, the great preponderance of union in pairs. The sporidia which are placed close to one another in whorls in *Tilletia*, *Entyloma*, *Urocystis*, and other genera unite, almost without exception, in pairs only, and when there is an odd sporidium, it usually does not conjugate, though its union with some pair might be easy, one might almost say would be very natural . . . These facts show that a change usually takes place in a pair after conjugation which renders a second union difficult or impossible, while it introduces the further development (*18*, *p. 181*).

Brefeld (*8*) on the other hand, considers the process of conjugation of the conidia as having no sexual significance whatever. He was the first to employ nutrient solutions successfully in prolonged cultures of the Ustilagineae, and observed the germination of a great many species. He reports bringing *Tilletia Caries* to the point of spore formation by his refined cultural methods. As a result of his intensive and tireless studies of these cultures he arrived at the following conclusion relative to the conjugation of the conidia: the fusing of the conidia never occurs so long as they are normally nourished and can multiply through budding; but it always takes place when the nutrient solution is exhausted (*8*, *p. 50*).

These differences of opinion of de Bary and Brefeld and their respective followers brought forth numerous theoretical discussions without furnishing new material to aid in solving the problem. With the development of modern cytological methods it became possible to study nuclear behavior in the Ustilagineae. The newer cytological results begin with the work of Dangeard (*11*) who observed nuclear fusion in the young spore.

According to this newer literature, we may consider as established the facts that the young spore cells, in the smuts so far studied, are binucleate, and on maturing become uninucleate by fusion, as maintained by Dangeard (*11*, *12*) Harper (*23*) Maire (*30*) Lutman (*29*) Rawitscher (*39*, *41*) and others. Furthermore there is unanimity of opinion that the mature uninucleate spore at time of germination produces a promycelium with uninucleate cells, in the case of the *Ustilago* group, and a multinucleate promycelium in the *Tilletia* group. In both the *Ustilago* and *Tilletia* groups the promycelia cut off uninucleate conidia, as maintained by Dangeard (*11*) Harper (*23*) Lutman (*29*) Rawitscher (*39*, *40*) and Paravicini (*34*).

Relative to the origin of the two nuclei in the young spore, there is yet insufficient evidence to sustain any of the suggested theories. Lutman (*29, p. 1208*) says that in *Ustilago Zeae* and *U. levis*, "the vegetative mycelium composed of multinucleated cells, breaks up into short segments containing one or two nuclei which pass into spores." In *Entyloma Nymphaeae* he observed that the mycelial cells are binucleate but he did not work out their origin. Rawitscher (*39*) states that in *Ustilago Maydis* the binucleate condition arises by the fusion of two uninucleate hyphal cells at the time of spore formation. In *Ustilago Carbo*, Rawitscher thinks that the binucleate condition arises at the time of the conjugation of the conidia or the mycelial cells themselves. Rawitscher (*40, 41*) extended his observations to *Tilletia Tritici*, *Cintractia Montagnei*, and *Urocystis Violae*, each of which he finds corresponds in nuclear history to *Ustilago Carbo*. Paravicini (*34*) in 1917 confirms Rawitscher in general, as does Kniep (*26*) in 1921, studying the whole life cycle of *Urocystis Anemones* as grown on artificial media.

The interpretations of the conjugation of the conidia may be summarized as follows: (a) as having sexual significance: de Bary (*17*) and Federley (*19*), and as also initiating the binucleate condition: Rawitscher (*39, 41*) Paravicini (*34*) Kniep (*26*) Bauch (*4, 5, 6*); (b) as a non-sexual or vegetative process: Brefeld (*8*) Dangeard (*11*) Harper (*13*) Lutman (*29*) Dastur (*13*) and Sartoris (*42*).

Dangeard and Harper did not observe nuclear migration during conidial conjugation. Federley and Lutman observed the migration of the nucleus from one conidium to the other. Federley thought that nuclear fusion occurred immediately in the conidia after nuclear migration. Lutman was undecided on this point. On the other hand Rawitscher, Paravicini, Kniep, and Bauch state that nuclear fusion never occurs in the paired conidia, but that this pairing initiates the binucleate condition.

As to the number of nuclei in the mycelial cells there is likewise a difference of opinion. Fisch (*20*) Schmitz (*43*) and Dangeard (*11*) were of the opinion that the mycelial cells of the smuts were multinucleate. Lutman (*29*) says that the *Ustilago* group alone possesses multinucleate cells. On the other hand, Maire (*30*) and Lutman (*29*) observed binucleate mycelial cells in the *Tilletia* group. Rawitscher (*39, 41*) Paravicini (*34*) and Bauch

(*4, 5*) maintain that the mycelial cells may be either uninucleate or binucleate. Dastur (*13*) Noble (*32*) and Sartoris (*42*) state that the mycelial cells may be either uninucleate or multinucleate.

In 1921 Anderson (*1*) described the development and pathogenesis of the onion smut. Employing methods similar to those of Whitehead (*49*), he succeeded in germinating fresh spores in sterile nutrient media, and grew the mycelium in pure culture.

Anderson states that the germination of the spores begins with a protrusion of a short hemispherical promycelium from which a whorl of branches grows out. These branches grow indefinitely without producing conidia. The growing saprophytic mycelium breaks up into short plump cells which Anderson thinks have the function of conidia and are probably of great significance in the dissemination of the smut organism. Anderson figures the early infecting hyphae and describes the parasitic mycelium as intercellular and showing both uninucleate and binucleate cells. He also describes and figures large haustoria but adds that these absorbing organs are not common. Anderson made a cursory study of spore formation in which he contributed no new data.

Anderson and Osmun (*3*) reported in 1924, that they placed smutted leaves of the onion in damp soil in test tubes in which they remained for three years. At the expiration of that time they found that the spores germinated by placing them on agar plates. The authors state that the germination began, within 2 to 5 days, by developing a simple unbranched tube from the central spore. A globose promycelium is not produced. Usually the germ tube remained simple and unbranched for some time, then branched only sparingly. As to just why the smut spores did not germinate in the moist soil, their natural medium, in which they remained for years, the authors offered no explanation. It is reasonable to suppose that the tissue of the onion leaves, permitted to remain in damp soil for three years, would soften to such an extent that the smut spores would be freed in the soil. In such a case it is questionable if one can say with certainty that the spores recovered from the soil after a period of three years are those of *Urocystis Cepulae*.

The germination process and the non-production of conidia in *Urocystis Cepulae*, as described by Anderson in 1921, are in

direct opposition to the observations of Thaxter (*47*) who, in 1889, described the development of the onion smut fungus. Whitehead (*49*) in 1921 reported his work on the life history and morphology of the onion smut and confirmed Thaxter's observations in general.

My study of the life history and nuclear phenomena in *Urocystis Cepulae* has dealt primarily with the following points:

(1) How and under what conditions do the spores germinate?
(2) What is the nature of the saprophytic stage, and how is it initiated?
(3) To grow the fungus in pure culture.
(4) How is the fungus able to maintain itself in the soil?
(5) To determine the nuclear phenomena of the saprophytic and parasitic mycelia.
(6) The origin of the fertile and sterile cells that compose the spore ball, together with the nuclear phenomena in each.

## MATERIAL AND METHODS

Since the onion smut is unfortunately not uncommon, an abundance of spores was available for experimental purposes. Dr. E. W. Olive, formerly of the Brooklyn Botanic Garden, and Dr. R. E. Kirby of Cornell University very kindly furnished me with a plentiful supply of spores for which I wish to express my sincere thanks.

Soil in a number of pots was infected with the smut spores. Onion seeds were sown at intervals so that seedlings at different stages of development were available at all times. The percentage of infection of the seedlings varied from about 30 to 100 per cent.

In order to study the parasitic stage of the organism, infected onion seedlings at various stages of development were fixed in different media. Flemming's weak chrom-osmic fixative gave the most satisfactory results. The material was imbedded in paraffin and sectioned three to five microns thick. The sections were stained with Flemming's triple and the iron-alum haematoxylin stains. The former proved to be the most useful. The septa between cells were brought out vividly, while the nucleoles, staining a brilliant red, served to distinguish the nuclei at all times. The Flemming triple stain was used with very short exposures and as little washing out as possible. The time and

the concentration of the stains were varied factors for the different stages of the life cycle of the organism

The spores were germinated in onion decoction, then plated out by the customary bacteriological method. The onion decoction was made by boiling 25 grams of sliced onion in one litre of distilled water until the liquid became opalescent. The decoction was then filtered, sterilized in the autoclave for 15 minutes at 15 pounds pressure, and the reaction adjusted to pH 7.1. The bean, carrot, potato, and onion media were prepared as follows: fresh green vegetables were washed thoroughly, tubed, and sterilized in the autoclave.

For the cytological study of the saprophytic mycelial cells and the germinating spores, a method proposed by Harper (*23*) was followed. Masses of mycelial cells were germinated in watch crystals, then, by means of a pipette, portions of the liquid containing the germinating mycelial cells were transferred into a drop of Flemming's solution. This fixing fluid containing the cells was 'stippled' on a slide previously covered with egg albumen. In the case of the germinating spores better success was obtained by coating a sterilized slide with a thin layer of onion agar; then placing spores from an unopened but mature smut pustule on the agar. The slide with the spores was then placed in a moist chamber. After several days the slide was stained in the usual way, the agar de-staining more rapidly than the fungus material. In this case, the iron-alum haematoxylin stain gave the best results.

### SOIL INFECTION

Onion seeds were sown in soil which previously had been infected with spores of *Urocystis Cepulae*. In about eight to ten days onion seedlings appeared above the surface. Owing to the peculiar method of germination of the onion seedling, the 'knee' is the first portion of the seedling to come through the ground. This is caused by the rapid elongation of the descending limb of the cotyledon, together with the growth of the primary root, which forces the seed end of the cotyledon upward through the ground. Since the seed coats are still attached to the upper end of the cotyledon and are dragged out of the ground within ten to eighteen days, the seedling at this stage of development has a characteristic appearance, similar to that of the figure 7. Within three to four weeks the first leaf makes its appearance.

It was observed that the cotyledons are susceptible to the attack of the smut fungus during their growth through the soil, as has also been reported by Thaxter (*47*) Sirrine and Stewart (*44*) Walker and Jones (*48*) Whitehead (*49*) Anderson (*1*) and others. It is also common to find infected seedlings remaining beneath the surface for periods longer than normal. Upon sectioning these cotyledons they were found to be severely attacked by the fungus. The mycelium had already permeated the tissues, thus apparently retarding their growth.

The presence of the smut in the onion seedling is first indicated macroscopically by a slight thickening of the cotyledon which also appears darker in color. If the plant is held up to the light, little opaque blotches can be seen in the cotyledon. These dark areas are the developing spore pustules which will within three to four weeks burst through the host tissue, owing to the rapid development of the spores within the sorus, and the drying up of the host tissue. These infected spots make their appearance most often below the 'knee,' though they sometimes occur above it, that is, nearest the attached seed-coat. A number of centers of infection may occur on the same cotyledon. When the infections occur only between the seed coat and the 'knee,' or just below the 'knee,' the upper end of the cotyledon withers up and apparently cuts off any further advance of the fungus in the host tissue. In such cases, the plants when the leaves have appeared seem to have wholly recovered from the early infection. This is especially true of the leek. Several pots containing infected soil were sown with leek seed. About 80 per cent of the cotyledons of the leek seedlings became infected. Apparently all of the seedlings survived the attack of the smut and grew vigorously for weeks after the disappearance of the diseased cotyledon. On the other hand, a great many of the onion seedlings are killed by the fungus during the early stages of growth. If the infection is not too severe, however, and the fungus happens to invade the zone of meristematic tissue from which develop all of the future leaves, the infections become in a sense systemic.

Of all the sections that I have examined, not one instance was observed in which the fungus had invaded the root system. The cotyledons are the only portions of the onion seedlings which permit the entrance of the fungus.

## SPORE GERMINATION

Thaxter (*47*) in 1889 succeeded in inducing the spores of *Urocystis Cepulae* to germinate from material gathered in summer and kept until the following January. Some of the smutted onions were spread out in a shed and left for six months, after which they were placed in moistened earth, saturated with water, and allowed to freeze for a week or more. After this treatment some of the smut, removed to a warm room and kept moistened, germinated. Thaxter also germinated the spores in onion decoction. His cultures, he says, swarmed with bacteria which soon destroyed them. Whitehead (*49*) in 1921, by freezing the spore pustules, then washing them in mercuric-chloride solution, brought about germination in water and in onion juice. Anderson (*1*) in 1921 described a method by which fresh spores of this fungus were germinated under sterile conditions in onion decoction and various kinds of nutrient agar.

I succeeded in germinating fresh spores in onion decoction and on onion agar as follows:

*Spore germination in onion decoction.* Mature but unopened sori, preferably those infecting the upper end of the cotyledon, were selected, and sterilized in mercuric-chloride solution (1–1000) for five minutes. They were then rinsed thoroughly in sterile distilled water to remove all traces of the mercuric-chloride solution. The pustules while in the sterile water were cut into small pieces which were transferred to cover glasses containing a small drop of sterile onion decoction (25 grams of onion to 1000 cc. of distilled water). The cover slips with the spores were inverted over a Van Tieghem cell and sealed with lanolin. Some of the cultures were placed in the refrigerator at a temperature of 18° C. Other cultures were kept at room temperature about 22°–25° C. In those cultures in which germination occurred, about 3 to 5 per cent of the spores germinated within four to seven days. However, only a small percentage of the cultures prepared in this manner showed germination, which may be attributed to several causes: first, some of the sori may not have been sufficiently matured; second, the mercuric-chloride may have penetrated some pustules and killed the spores; third, there is a possibility that the sealed cells lacked the necessary amount of oxygen.

*Spore germination on onion agar.* The spore pustules were

sterilized and rinsed in water. The sterilized sori were transferred to sterile distilled water and the pustules crushed in order to scatter the spores in the water. Then the spores were transferred by means of a pipette to thin onion-agar plates, all glassware and implements being sterile. The plates were kept at a temperature of 18°–25° C. A much higher percentage of germination occurred on the onion agar plates than in onion decoction: often 70 to 80 per cent of the spores germinated. Some lots of spores taken from the same pustules germinated better on onion agar plates than in Van Tieghem cells in onion decoction. When the spores in onion decoction, sealed in Van Tieghem cells, had ceased to germinate, the following experiment was performed: the spores from two of the cultures in Van Tieghem cells were spread on onion agar plates. The other onion decoction cultures were permitted to remain sealed in the Van Tieghem cells, as controls. On the onion agar plates, about 80 per cent of all the spores so spread out germinated, while in the onion decoction cultures no further germination occurred, that is, only about five per cent of the spores had germinated, as described above.

All the spores do not germinate at the same time. At the end of the third day a few germ tubes were noticed. Additional spores began to germinate from day to day, up to and including the fifteenth day. This progressive germination is also evidenced by the development of the mycelial weft about each spore. Some are visible to the naked eye, about one millimeter in diameter, while others appear as minute white dots. Examined microscopically the plate shows small tufts of mycelia, grading down to the spores just beginning to germinate. An agar plate from ten to fifteen days old presents spores in all stages of germination.

Anderson (*1*, *p. 108*) thinks that "the period of preparation for germination differs for different spores, so that the germination period extends over many months, possibly years."

Germination begins by the protrusion from the central spore of a somewhat spherical hyaline promycelial vesicle. At first this vesicle is about the size of the neighboring pseudospores of the spore ball. Growth takes place rapidly and its size increases until it may measure six to ten microns in diameter (FIG. 1, PLATE 3). This vesicle is apparently the promycelium. It varies considerably in size; in some cases it remains extremely small. Soon the sub-spherical promycelium buds out a tube,

which, from its subsequent development, we may characterize as a determinate hyphal branch (FIG. 1, PLATE 3). This is immediately followed in succession by other similar hyphae (FIGS. 2–4, PLATE 3). The number of branches that arise from the promycelium varies: four, six, and eight branches were observed in the greatest number of cases. Cross walls dividing these hyphae into cells soon appear (FIG. 3, PLATE 3). The cells nearest to the promycelium in turn bud off cells in a characteristic fashion indicated in FIGS. 3, 4, 5, PLATE 3. These hyphae develop immediately into mycelia of indeterminate capacity for growth which branch profusely so that within twelve to eighteen hours a dense weft of mycelium is produced about the spores, either in onion decoction or on the onion agar plate. After two or three days, the older cells of the mycelium tend to round up and become more or less separated from each other: at the same time, rapid growth occurs at the periphery of the mycelial mat or ball.

Thaxter (*47, p. 142*) described the process of spore germination of *Urocystis Cepulae* as follows:

> The germination consisted in the production from the central resting spore of usually a short hypha of germination, which commonly branched more or less and produced, terminally or laterally, small secondary spores, the so-called sporidia.

Whitehead (*49*) states that at the time of germination one, or rarely two germ tubes are produced, which cut off laterally minute oval sporidia. He says that these sporidia multiply by budding but do not conjugate. Anderson (*1, p. 110*) did not observe the production of conidia. He says, "No conidia have been observed on the promycelium or its branches or any where else throughout the development of the organism." My observations of spore germination of *Urocystis Cepulae* corroborate, in general, those of Anderson.

Since the spores of *U. Cepulae* do not germinate until the third to the seventh day, if the cultures are in the least contaminated, they are simply overrun with bacteria and molds before the germination of the spores begins. This is especially true when a nutrient solution of the nature of onion decoction is used. The use of pure cultures, as obtained by Anderson and myself, is imperative if the normal germination of the spores is to be observed.

### NUCLEAR PHENOMENA DURING SPORE GERMINATION

Apparently heretofore the nuclear phenomena in the germina-

ting spores of *Urocystis Cepulae* have not been observed. In order to accomplish this, the following method was employed: spores were taken from unopened, mature, but sterilized sori and placed on sterile slides which previously had been coated with a thin layer of onion agar. The slides were then placed in a sterile moist chamber. After the spores began to germinate they were fixed *in situ* in Flemming's weak solution; then stained in the usual way in iron-alum haematoxylin stain. By this process the nuclei in the hyphal cells were clearly differentiated. The first divisions of the nucleus take place apparently in the spore, as has been described for *Urocystis Anemones* by Kniep (*26*), for *Tilletia Tritici* by Rawitscher (*40*, *41*), and for *Urocystis Tritici* by Noble (*32*). Owing to the thick opaque spore wall and the surrounding envelope of sterile cells, the behavior of the nucleus within the germinating spore was not discernible even in thoroughly bleached preparations. In FIGS. 1 and 2, PLATE 3, the early stages of germination are represented. The nuclei pass from the spore through the short promycelium into each of the promycelial branches only after these structures have attained a certain development, for which reason neither the hyaline spherical promycelium nor the young hyphae as yet contain a nucleus. In slightly older stages as represented in FIG. 3, PLATE 3, the primary determinate hypha is already divided by a septum into two cells, each cell containing a nucleus measuring less than one micron in diameter. The cell next to the promycelium has begun to bud out another cell at its upper end. Hyphae are budded off by the promycelium in succession, as described above. As shown in FIGS. 4 and 5, PLATE 3, each hypha divides into cells, each of which contains a single nucleus. Each nucleus appears as a spherical body with chromatin content and a definite rounded nucleole. With the Flemming triple stain, the chromatin material was stained a light purple, and the nucleole a brilliant red.

Conjugation between the branches of the promycelium, as described by Kniep (*26*) for *Urocystis Anemones* and by Rawitscher (*41*) for *U. Violae*, was not observed in *U. Cepulae*. The primary hyphae continue to grow, and develop into uninucleate mycelial threads. Conidia, as noted above, are not produced in *U. Cepulae* in any stage of germination.

## COMPARISON OF THE PROCESS OF GERMINATION AND THE ATTENDING NUCLEAR BEHAVIOR OF UROCYSTIS CEPULAE WITH THAT OF OTHER SPECIES OF UROCYSTIS

*Urocystis occulta* Wallr. Kühn (*27*) states that from two to three germ tubes of different sizes are formed. On the stronger promycelium two to six primary conidia appear. Wolff (*51*) in 1873 observed that the conidia germinate without falling off. According to Brefeld (*9, p. 175, fig. 1, pl. 11*) spores of this species produce a promycelium of varying length, which at its apex produces a whorl of four to six branches. These increase in length by apical growth. Both the promycelium and the branches become septate and poorer in protoplasmic content. Finally only the tips of the branches contain active protoplasm. Conidia, according to Brefeld, are never formed. Nutrient solutions did not change the form of this growth nor lead to the production of conidia. The description of McAlpine (*31*) in 1910 corresponds to that of Brefeld, with the exception that he calls the branches of the promycelium conidia.

*Urocystis Filipendulae* Tul. The whole germination process as described by Brefeld (*9, p. 176, fig. 3, pl. 11*) is quite similar to that observed by the writer for *U. Cepulae:* a very short promycelium with long branching mycelial outgrowths that produce no conidia.

*Urocystis Tritici* Koern. This species is distinguished from *U. occulta* only by the fact that the spores from the host of one will not infect the host of the other. The germination of the spores of *U. Tritici* has been described by McAlpine (*31*) and Noble (*32*). The latter states that the young promycelium contains a variable number of nuclei derived from the single nucleus of the spore. His figures show that two to four sporidia develop at the apex of the promycelium. One nucleus passes into each of the sporidia. The sporidia while attached to the promycelium produce slender germ-tubes which become binucleate by the migration and division of the single nucleus. In a few cases Noble observed the conjugation of conidia, and states "It appeared as if the single nucleus of each conjugating conidium migrated into the fusion germ tube" (*p. 482*).

*Urocystis Anemones* (Pers.) Winter. Fischer von Waldheim (*21, 22*) Plowright (*35*) Brefeld (*9*) Paravicini (*34*) and Kniep (*26*) have observed the germination of the spores of this species.

According to Brefeld a short thick promycelium develops which, in turn, produces three to four long mycelial threads (*p. 176, fig. 2, pl. 11*). Paravicini states that the branches of the promycelium fall off easily and by division produce a chain of uninucleate mycelial cells. The mycelial cells, he thinks, become binucleate by the dissolving of the cell wall separating two of these cells. However, Paravicini's figures do not convince one that this is the case. Kniep (*26*) reports growing saprophytically the complete life cycle from smut spore to smut spore of *U. Anemones* on 0.5 per cent malt extract media. He states that the fusion nucleus divides twice while in the spore. A short promycelium is developed which soon buds out a whorl of three or four branches. The nuclei now migrate from the spore into the promycelium. If but three branches are present, each of the nuclei passes into a respective branch, while the fourth nucleus remains in the stalk cell (promycelium). Conjugation occurs between these unicellular units so that two branches will contain two nuclei while the other two elements will be empty. The binucleate element increases in length by apical growth as described for *U. occulta*.

*Urocystis Violae* Sow. Prillieux (*38*) Dangeard (*11*) Brefeld (*9*) Paravicini (*34*) and Rawitscher (*41*) have all observed the germination of this species. Rawitscher states that at the time of germination of the spores, a multinucleate promycelial tube is formed, at the tip of which seven or eight conidia are produced. One nucleus migrates into each conidium. The conidia conjugate by slender conjugation canals through which the nucleus from one conidium migrates to the other. The binucleate conidia grow in length as Kniep observed for *U. Anemones*.

*Summarizing:* In *Urocystis occulta* and *U. Filipendulae* the process of germination, aside from the nuclear phenomena, which are still unknown, agrees in general with that of *U. Cepulae*. In *U. Anemones*, *U. Violae*, *U. Tritici*, and *U. Cepulae*, the fusion nucleus divides within the spore. The nuclei then migrate through the promycelium to the promycelial branches, into each of which one nucleus passes, forming uninucleate branches. In all of the above named six species of *Urocystis*, the promycelial branches germinate and grow in length while attached to the promycelium.

Conjugation of the promycelial branches (conidia) occurs in

*Urocystis Anemones*, *U. Violae*, and *U. Tritici;* while in *U. Cepulae* conjugation between promycelial branches does not take place, but the hyphae grow at once into a mycelium with uninucleate cells, without the production of conidia.

The promycelium of the species of this group varies in shape and size. It is sub-spherical in *U. Cepulae*, very short in *U. Filipendulae* and *U. Anemones*, tube-like in *U. occulta*, *U. Tritici*, and *U. Violae*.

ISOLATION AND CULTURAL CHARACTERISTICS OF THE MYCELIUM

Spores from mature pustules were spread on thin onion agar plates, after which the spores were located by means of the microscope and marked. In from ten to fifteen days the mycelium developed from a single spore will be visible to the unaided eye as a tiny white dot. These tiny white mycelial balls were transferred to various media. Luxuriant growths of mycelium occurred on onion agar, sterile bean, potato, carrot, and sterilized fresh onion.

*Cultures on onion agar.* A tiny white ball of mycelium which had developed from a single spore was transferred from the plate to an onion agar tube fourteen days after the spore had germinated. After forty-eight hours growth on the onion agar, it was photographed (FIG. 1, PLATE 1). At this stage of growth it is a spherical mass of mycelium whose hyphal branches extend outward and radially in all directiions, and is snow-white, due to the inclusion of air between the hyphae.

A growth of three and a half days is shown in FIG. 2, PLATE 1. The structure of the mycelial weft and the growth habit of the hyphae is strikingly shown. At this stage it has a cottony appearance.

As the growth continues and the mycelial weft spreads more or less equally in all directions on the surface of the agar, simultaneously the mycelial threads pile up and intertwine so that after ten days' growth the culture appears as is shown in FIG. 3, PLATE 1. The edges of the cultures are somewhat sharply marked, but with a hand lens one can detect a fringe of hyphae extending outward beyond the denser mass. Cultures at this age ranged in diameter from three to six millimeters and in height from one to two and a half millimeters. The surfaces of these cultures now show nodular or ridge-like elevations which

frequently arise at first in or about the center. Sometimes they occur over the entire surface of the culture. At this stage the cultures become a light gray.

After 16 days' growth the surface elevations have developed into pronounced wrinkles as indicated in FIG. 4, PLATE I. In this figure a delicate, compact, even fringe of hyphae is visible at the edges of the mass. Not all of the cultures of this age show such a pronounced fringe. The color has become darker with a gray sheen which is apparently due to the development of short aerial hyphae. FIG. 5, PLATE I, represents a growth of 18 days which had been transferred to onion agar from a culture growing on a bean. FIG. 6, PLATE I, represents a culture 61 days old. As growth proceeds the wrinkles become more like ridges with crests of higher elevation than in the preceding cultures. The gray changes to a brownish tinge. As the agar loses its water content the mycelium shows zonal growth more clearly.

*Cultures on sterilized potato.* The growth on fresh sterilized potato resembles that on onion agar, but is considerably more rank. After seven days' growth the culture measured 6.5×7 mm. and 2.5 mm. thick. It is then a compact dense mat of mycelium, snow-white, with definite edges of irregular outline (FIG. 7, PLATE I). The surface is also irregular, suggesting the development of the wrinkles which will soon make their appearance. The culture continues to grow vigorously for about four weeks at which time a heavy rank growth covers the upper surface of the potato, showing the characteristic wrinkles described for the growth on onion agar. From this time on, the culture becomes less vigorous, seems to shrink together and dies within a few months. FIG. 8, PLATE I, shows a culture 51 days old. The mycelial mat is thin, dry, and quite flat. The crests of the remaining wrinkles crack open. The culture is white throughout.

The cause of the declining growth and ultimate death of the fungus is apparently due to some change in the potato. If transfers are placed on potatoes three months after sterilization, there occurs but a scanty growth, which soon dies.

*Cultures on sterilized onion.* Growth on sterilized onion at first was exceedingly slow. Instead of spreading, as was the case on onion agar and on potato, the mycelium developed a tiny compact ball about one millimeter in diameter, hygrophanous, and gray. A few mycelial threads extending from the

ball start other centers of growth, each center subsequently rounding up into a little sphere, similar to the first. After a growth of two weeks a number of these little mycelial balls were present in the immediate vicinity of the original transfer. As evaporation of the water goes on the mycelial balls increase in size, pile up over each other, and finally coalesce in such a manner that a mycelial mat with uneven and wrinkled surface is formed. The crests of the higher elevations become white while the mass of the mycelium remains hygrophanous and gray (FIG. 9, PLATE 1).

An attempt was made to induce spore formation on a living onion bulb. An onion was pared under aseptic conditions and infected with the fungus, then placed in a sterile moist chamber. In a few days the fungus showed signs of growth. Simultaneously the onion tissue began to darken in the immediate vicinity of the infection, as if the tissue were being killed. Finally the fungus produced the characteristic mycelial mat with wrinkled surface (FIG. 10, PLATE 1). At no time was there any appearance of spore formation.

*Cultures on sterilized bean pods.* Transfers were made from the onion agar plate to sterilized bean pods. Growth was vigorous from the beginning. At first the culture shows a snow-white weft of mycelium, as observed on the onion agar . on the potato. In about a week it appears as a compact mat of mycelium with definite but irregular edges. The growth on the bean distinguishes itself from that on other media by the fact that the fungus tends to pile high in a mass, layer upon layer, as shown in FIG. 1, PLATE 2, which shows a seventeen day old culture. As growth progresses the culture extends itself partly around the bean, as indicated in the 51 day old culture in FIG. 2, PLATE 2. At this stage the wrinkles are larger and coarser, a specific feature of the bean cultures. The central mass of the culture ranges from dark gray to brown, while in the periphery a delicate white weft of mycelium is evident. Zonal growth next appears, a certain indication of the appearance of conditions unfavorable for the fungus. Secondary mycelial mats often occur, started probably by bits of hyphae from the primary mat being scattered in handling the culture.

In no case has the writer ever observed conidia or spore formation of any kind on culture media.

*Cultures on sterilized carrot.* Growth on the carrot resembles that on the sterilized onion. After the carrot becomes drier the fungus takes on the characteristic form of a mycelial mat. FIG. 4, PLATE 2 represents a 25 day growth. Soon little rope-like ridges appear, which anastomose as indicated in FIG. 5, PLATE 2, a 47 day old culture. As the culture ages, the ridges become proportionately enlarged and run together, thus producing a more even surface than is seen on the onion agar, potato, or bean cultures (FIG. 6, PLATE 2).

*Cultures on liquid nutrient media.* Bits of the mycelium were transferred to tubes of 2 per cent cane sugar solution, and tubes containing onion decoction. The growth was similar in each case. The mycelium formed little balls which never exceeded more than one to two millimeters in diameter. If the mycelial weft becomes attached to the sides of the test tube a slight growth of white mycelium results.

In résumé it can be said that very young cultures of *Urocystis Cepulae*, on solid media, present a cottony appearance. In older cultures when the water content is not too high, the wrinkling of the surface is a constant feature. The shape and form of the wrinkles vary according to the kind of media used and the age of the culture. FIG. 7, PLATE 1, and FIG. 1, PLATE 2, represent cultures of the same age but grown on different media, showing characteristic variations in appearance. FIG. 6, PLATE 1, and FIGS. 2 and 6, PLATE 2, are cultures of different ages grown on agar, bean, and carrot, respectively, and likewise show that the wrinkles vary in form, size, and direction of growth. In a liquid medium the culture is characterized by the formation of small mycelial balls.

The vitality of the fungus is not reduced by transfers from one culture to another, or, so far as tested, from one kind of medium to another. Apparently cultures can be continued indefinitely. FIG. 5, PLATE 1, shows a culture which was transferred from bean to onion agar, this representing the fourth transfer from the agar plate. FIG. 1, PLATE 2 was transferred from carrot to bean, and likewise represents a fourth transfer.

The growth habit of *Urocystis Cepulae* in culture is quite distinctive in appearance when compared with cultures of certain other fungi. Blakeslee's photographs of *Mucor Mucedo* (7, *fig. 55*) and of *Phycomyces nitens* (*fig. 52*) show that the hyphae

spread out evenly over the medium, forming a loose mycelial weft on the surface of the plate, with an abundance of aerial hyphae. On the other hand cultures of yeast obtained by Lindner (*28*) are quite similar in appearance to those of *U. Cepulae*. Certain of Lindner's cultures (numbers *68*, *70*, *plate 85*) show that the yeast cells pile up on each other and spread out, forming a thick mat with a definite wrinkled surface, comparable in appearance to my FIG. 4, PLATE 1, and FIG. 6, PLATE 2 of *U. Cepulae*.

The cultural characteristics of *Tilletia Tritici* as shown by Sartoris (*42*, *figs. 2, 4, plate 39*) are different from those of *Urocystis Cepulae*. Sartoris says

> Two or three weeks after germination of the chlamydospores on oatmeal agar a mycelial layer appears. This spreads until it covers the surface of the agar by its radial growth. It is white at the surface of the layer and of a fine leathery texture. It is about 0.5 to 1 cm. in thickness at the center of the mat: here also it is thickest and forms a peak (*p. 625*).

The mycelium of *Ustilago Zeae* grown on glycerin agar by Sartoris forms a thin mycelial mat. A study of his photograph (*fig. 3, plate 39*), gives the impression of nodular elevations formed on the surface, but quite different in appearance from the cultures of *U. Cepulae*.

Potter (*36*) grew in pure culture the mycelium of *Sorosporium Reilianum*, the causal organism of head-smut of sorghum and maize. He found that the organism developed well on malt-extract, beerwort, and carrot agars. A synthetic dextrose agar also proved to be a favorable medium. The growth on carrot agar at 30° C. produced a thick mycelial mat whose surface bore numerous wrinkles that anastomosed freely (*fig. 1, plate 34*). His *figs. 2, 3, plate 34* on glucose and on carrot agar respectively Potter states, "show the characteristic rugose growth." Thus the cultural characteristics of *Sorosporium Reilianum* are indistinguishable in appearance from those of *Urocystis Cepulae*.

Anderson (*1*) grew *U. Cepulae* on a large number of culture media, and describes its growth on onion agar as follows:

> The growth begins with a dense white felt . . . after about a week wrinkles begin to appear near the center, and these spread and become sharper and the irregular ridges more elevated with age. . . . The crests of the ridges become hygrophanous and gray (*p. 113*).

My observations of the cultural characteristics of *U. Cepulae* in general corroborate those of Anderson. Anderson states that

"the convoluted gray growth on onion agar is perhaps the best diagnostic cultural character of the species" (*p. 114*). Certainly this is true when cultures of *U. Cepulae* are compared with cultures of fungi like the Mucorineae. But it would be difficult or almost impossible to distinguish cultures of *U. Cepulae* from certain yeast cultures or cultures of *Sorosporium Reilianum*, as noted above.

### THE NUCLEAR PHENOMENA IN THE SAPROPHYTIC MYCELIUM

In order to study the nuclear phenomena in the young mycelium, spores were placed on a slide previously coated with agar, and permitted to germinate under sterile conditions. In ten to fifteen days little tufts of mycelia in various stages of growth developed about the spores. These little tufts of mycelia were fixed *in situ* in Flemming's weak solution and stained in iron-alum haematoxylin and Flemming's triple stain.

The microscopic appearance of the mycelium varies with the age of the cultures. In examining the little tufts of mycelium on the onion agar, mycelial threads were observed to have spread out over the surface of the agar to a distance of about 100 microns from the periphery of the culture mass. In some of these hyphal threads the basal cells soon become empty while the tips are full of dense homogeneous protoplasm. It is difficult in many cases to find any connection between the hyphal tips thus isolated and the main culture. These isolated hyphal tips branch and become the center of new growths which soon fuse with the older mass.

These young cultures are composed of slender branching hyphae measuring from one to two microns in diameter, with rather homogeneous cytoplasm. The hyphae, in fact, do not differ materially from the hyphae as described at the time of spore germination, represented by figs. 4 and 5, PLATE 3. In all young cultures thus examined, the mycelium was observed to be composed of uninucleate cells. The nuclei are definitely differentiated by the stains, each nucleus containing a well-defined nucleolus.

As noted, conidia are not produced by any part of the young mycelium. Paravicini (*34*) reports in the case of *Urocystis Anemones*, which species he says produces no conidia, that nuclear migration from one hyphal cell to an adjacent cell of

the same hypha may occur. This nuclear migration, according to Paravicini, is made possible by the gelatinization of the septum between the two cells. Thus a binucleate condition is initiated in the hyphae, which he thinks is the origin of the binucleate condition that is found at spore formation. A very careful examination of the saprophytic mycelial cells clearly shows that no such nuclear migration occurs in *Urocystis Cepulae*. The cells of the saprophytic mycelium are uninucleate from the beginning and remain so throughout their existence.

For the study of older mycelia two methods were employed: (1) mats of mycelia growing on agar were sectioned and stained; (2) small portions of mycelia were broken up in water and stippled on the slide.

Small blocks of agar containing the eighteen to twenty day old mycelium were fixed and imbedded in paraffin. These blocks were sectioned 3–5 microns thick, and stained with Flemming's triple and iron-alum haematoxylin stains. In such sections it could be clearly observed that the hyphae of the older cultures presented a different appearance from that of younger growths. Some of the hyphal threads that had penetrated the agar could be traced continuously into the aerial hyphae in the center of the culture mass. These hyphae were considerably larger than the surrounding hyphae, measuring 2–3.5 microns in diameter (FIG. 9, PLATE 3). Their cells appear well nourished and the cytoplasm contains refractive granules and stains densely (FIG. 7, PLATE 3). In the same section can be seen other hyphae whose cells tend to be rounded at the ends and constricted in the middle, giving the entire hyphal thread the appearance of a chain of beads. Some of these dumbbell shaped cells are rich in cytoplasm (FIG. 6, PLATE 3). In the very old or more desiccated cultures the cytoplasm was reduced to a primordial utricle with thin strands of cytoplasm stretching across the cell from side to side (FIG. 10, PLATE 3). On either side of the nucleus of these cells, towards the end, are large vacuoles. In each case, the nucleus occupied the central portion of the cell in the region of the constriction, surrounded by a scanty amount of cytoplasm.

The method of branching of the hyphal cells is characteristic. Most frequently at the distal end of the cell just beneath the septum, a cytoplasmic extrusion occurs (FIG. 12, PLATE 3).

This growth may continue until it is almost or quite the length of the mother cell, before a nucleus appears in it (FIG. 4, PLATE 3). In some cases the nucleus migrates into the branch and there divides (FIG. 9, PLATE 3). In other instances two nuclei were observed in the mother cell and none in the branch. Finally a nucleus is present in each portion, and a septum is laid down at or near the base of the branch. The axis of growth of the branching cell forms an angle of about 70° with the long axis of the mother cell, and gives to the mycelium a characteristic appearance. The branches may at times be given off at the lower end of the cell as shown in FIG. 12, PLATE 3.

TEXT-FIG. 1. A photomicrograph of germinating hyphal cells in onion decoction culture 18 hours. Each cell has produced a thin germ tube at one or both ends. × 600.

It was observed that any tiny piece of mycelium, even the individual cells of the mycelium themselves, are capable of starting new growths on the various media. Bits of mycelium from the cultures on bean, on carrot, and on onion, were transferred to a few drops of sterile water in a hollow ground slide. The mycelium was stirred and shaken in such a manner as to separate the cells from each other. The individual cells appeared as represented in FIG. 6, PLATE 3. These cells were then spread on thin agar plates. With the aid of the microscope individual cells were located on the onion agar and marked so

that their subsequent development could be followed. Every cell observed 'germinated' (see TEXT-FIGURE 1), and produced within fifteen to eighteen days a mycelial growth visible to the unaided eye, in a manner similar to that described above for the germinating spore. The mycelia resulting from such germinating hyphal cells are shown in FIG. 7, PLATE 2. For comparison, FIG. 8, PLATE 2 shows mycelium which has developed directly from a mass of germinating spores. The general appearance of the mycelia resulting from germinating hyphal cells, and that of the mycelia developing from a mass of germinating spores is the same.

In another experiment, bits of mycelia were broken up into their cellular elements, as described above. Then by means of a sterile pipette these hyphal cells were sprayed over a sterilized bean pod. Apparently wherever a mycelial cell settled upon the bean pod a growth resulted as evidenced by the distribution of the hyphal mats shown in FIG. 3, PLATE 2. This culture was 51 days old at the time of photographing. A number of the mycelial colonies have fused. On the upper portion of the bean pod a well developed mycelial mat with the characteristic wrinkled surface is formed.

In order to follow the nuclear phenomena in germinating hyphal cells such as are shown in TEXT-FIGURE 1, hyphal cells broken apart were placed in a hanging drop of onion decoction and sealed in a Van Tieghem cell. The usual precautions to keep the culture pure were observed. After eighteen hours a drop of Flemming's weak solution was mixed with the culture and the preparation was allowed to stand for thirty minutes. The fixing solution, together with the mycelial cells, was then stippled on slides which had previously been coated with egg albumin. The slides were then placed in 50 and 70 per cent alcohol to coagulate the albumin. By this method numerous hyphal cells remained attached to each slide and were subsequently stained by Flemming's triple and iron-alum haematoxylin stains. In all the slides examined apparently every one of the dumb-bell shaped cells 'germinated' by budding out at one end a slender cytoplasmic tube (FIGS. 8, 11, 14, PLATE 3). The nucleus soon migrates into the tube and divides as indicated in the upper figure of FIG. 8, PLATE 3. A septum is laid down between the two nuclei as indicated in the lower figure of FIG.

8, PLATE 3. Some of the cells may bud out slender hyphal tubes at both ends. In these cases, the nucleus still remains in the original cell (FIG. 14, PLATE 3). In other cases, the nucleus and cytoplasm appear to migrate into the germ tube (FIG. 8, PLATE 3) leaving the original hyphal cell empty. Subsequent branching of the hypha takes place, as previously described. The mycelium arising from these hyphal cells is composed of uninucleate cells, as is the mycelium which had its origin from a germinating spore.

Apparently each mycelial cell of the saprophytic mycelium may function as an oidial spore, serving to propagate and disseminate the fungus in the soil.

Dessication does not kill the mycelial cells, as is indicated by the following experiment. Cultures which had been growing on thin agar plates were permitted to dry. After four weeks, when the plates were air dry, portions of the mycelia were examined and observed to be composed of hyphal threads which had the appearance of oidial chains, due to the rounding of the cells at their ends and a constriction in the middle (FIG. 10, PLATE 3). Such cells measured about $6 \times 1.5$–2 microns. Some of this dessicated mycelium was shaken in water to separate the cellular elements. These separated, dessicated mycelial cells were placed in onion decoction and other media favorable to their growth. In eighteen to twenty-four hours they budded out slender germ tubes, as described above. From such dessicated cells, cultures were developed similar to the one which is represented in FIG. 7, PLATE 2.

The persistence of the fungus in the soil was shown by the following experiment: Pots containing infected soil in which onion seedlings infected with the smut fungus had previously been grown, were set aside in March, 1921, in a dry room, and exposed to the sun. This soil was 'air-dry' throughout the summer of 1921 until November of that year, at which time the soil was moistened and sown with onion seeds. In a few weeks about 60 per cent of the seedlings showed smut infection. Controls showed no infection.

### INFECTION OF THE HOST WITH MYCELIA FROM CULTURES

To test the ability of the saprophytic mycelium to infect the natural host, the following experiments were made:

*Experiment A.* Soil was placed in six large test tubes which

were then plugged with cotton and sterilized in the autoclave for thirty minutes under eighteen pounds pressure. Onion seeds were sterilized for five to ten minutes in mercuric-chloride solution, then washed in sterile water and placed in a moist sterile chamber. When the onion seeds germinated, healthy vigorous onion seedlings at different stages of development were transferred to the sterile soil in three of the test tubes, numbered one to three. To these three test tubes were transferred mycelium from the various cultures, in each instance packing it around the seedlings as closely as possible. The other three test tubes were kept as controls, some sterilized onion seeds having been planted in each of them. Results are shown in TABLE 1.

TABLE 1

| *No. of test tube* | *No. of seedlings* | *Infected* | *Not infected* | *Controls* |
|---|---|---|---|---|
| 1 | 12 | 12 | 0 | 0 |
| 2 | 12 | 3 | 9 | 0 |
| 3 | 12 | 6 | 6 | 0 |
| Total | 36 | 21 | 15 | 0 |

*Experiment B.* Soil was placed in pots and sterilized by dry heat for four hours at a temperature of 178° C. Ten onion seedlings were removed from the sterile moist chamber to each pot. The onion seedlings were then covered with bits of mycelium which had been broken up in sterile water and a half-inch layer of sterile soil placed over the seedlings in the pot. This top layer was then thoroughly moistened with sterile water in which a considerable amount of the fungus mycelium had been placed. Sterile water was used to keep the soil moist. Pieces of glass were placed over the top of each pot. Controls were run with each. The results are shown in TABLE 2.

TABLE 2

| *Pot* | *No. of seedlings* | *Infected* | *Not infected* | *Controls* |
|---|---|---|---|---|
| 1 | 10 | 7 | 3 | 0 |
| 2 | 10 | 10 | 0 | 0 |
| 3 | 10 | 3 | 7 | 0 |
| 4 | 10 | 2 | 8 | 0 |
| 5 | 10 | 10 | 0 | 0 |
| 6 | 10 | 5 | 5 | 0 |
| Total | 60 | 37 | 23 | 0 |

Results of the two experiments:

| | *Infected* | *Not infected* |
|---|---|---|
| 6 test tubes | 21 | 15 |
| 6 pots | 37 | 23 |

*60.5% infected.*

The possibility that smut spores were carried over with the inoculum is hardly conceivable when one considers that most of the cultures from which the mycelium was taken were each a product of a single spore. Furthermore, some of the inoculum was mycelium from the second and third transfer from the original spore or spores that produced the mycelium. It is interesting to note here that some of the mycelium that was placed in the soil in *Pot 2* was recovered after twelve hours. This mycelium was mounted on a slide in the usual way and stained. Some of the cells had begun to germinate and are represented in FIG. 13, PLATE 3.

It seems probable that infection in the field may commonly take place from mycelia or hyphal cells, rather than more directly from the germinating spore.

In the above experiments it was observed that the period elapsing between the time when the young seedlings were inoculated with mycelial cells and the first appearance of the dark pustules varied from nine to fourteen days in the case of the young cotyledons. Seven days later the mature pustules were formed. Thus the parasitic stage of the onion smut, in cases when the seedling only is infected, may cover a comparatively short period from the time of infection until complete spore formation, that is, from three to four weeks. In the case of systemic infections, this period of time is considerably lengthened, the pustules not making their appearance until the leaves are well-developed.

## THE CHARACTER AND NUCLEAR PHENOMENA OF THE PARASITIC MYCELIUM

The parasitic mycelium of *Urocystis Cepulae* is intercellular throughout its existence. The vegetative hyphae can be observed to run singly between the cells of the host, or there may be three or four mycelial threads running parallel in the intercellular spaces. These hyphae are delicate slender tubes, most of the cells of which are long and slim, measuring 20–25 microns in

length, to 1–1.5 microns in diameter. They are most frequently observed running parallel to the long axis of the cells of the host tissue. As one follows these hyphae up and down, nests of mycelia are found which have been formed by frequent branching of the hypha. With Flemming's triple stain the cellular boundaries are well brought out. Branches of the hyphal cells arise usually at the end, and just beneath the septum (FIG. 4, PLATE 3) as described for the saprophytic hyphae. The cytoplasm is homogeneous. The nuclei are extremely small structures throughout the parasitic stage of *U. Cepulae*, averaging in size from 0.5 to 0.8 microns in diameter, while the nucleoles, of course, are much smaller. By carefully manipulating the Flemming triple stain the whole nucleus will stain a light purple while the nucleole will be a brilliant red, the cell septa staining a dull red. However, in most cases, because of the affinity of some of the cells for the gentian violet, de-staining is advisable until only the nucleoles and cell septa stand out prominently.

Certain of the hyphal cells were observed to contain but one nucleus (FIGS. 4, 5, PLATE 4). These uninucleate cells were always observed to be at some distance from the young developing sorus. I have followed with extreme care the tortuous route of these hyphae as they wind their way over and around the host cells, but in spite of diligent search I was unable to find any trace of cell fusion. Haustoria or special feeding branches do not occur in *U. Cepulae*. In one case, two clavate hyphae were observed to have penetrated the cell wall and extended some distance within the cell. In one other case, an unusually large hypha was observed to have penetrated through one cell, and apparently was making its way into the adjoining host cell. Such cases are unusual and are to be considered as variations from the usual growth habit of the organism. Whitehead (*49*) observed hyphae of a simple type, which he called haustoria, within the host cells, and says that they are similar in appearance to the intercellular hyphae. On the other hand, Anderson (*1*) has described and figured 'large complicated haustoria.' He adds, however, that "in some infections none could be found, while in others they are fairly common." (*p. 126*). Neither Whitehead nor the writer observed such complex haustorial structures as reported by Anderson.

As one traces the mycelium towards a sorus, the hyphae

running in the intercellular spaces increase in number by profuse branching; they may twine around each other forming strands of mycelium which force the host cells farther apart. The cells of these hyphae are predominantly binucleate. The two nuclei lie in the center of the cell a short distance apart (FIGS. 1–3, PLATE 4). Numerous transverse branches from the mycelial strands penetrate radially the spaces between the mesophyll cells of the host. The vascular bundles are never invaded by the fungus. In the region where the sorus develops, the host tissue is thoroughly infiltrated with the hyphae of the parasite. The hyphal cells of the sorus primordia are all binucleate. This observation agrees with those of Lutman (*29*) for *Urocystis Anemones*.

Thus the hyphal cells of the parasitic mycelium are at first uninucleate but become binucleated as they approach the sorus primordium where all the cells of the sporogenous hyphae are binucleate (FIGS. 6, 7, PLATE 4). These sporogenous regions constitute the great bulk of the parasitic mycelium and thus it follows that the great mass of the parasitic hyphal cells of *Urocystis Cepulae* are binucleate. Lutman (*29, p. 1213*) reports in the case of *U. Anemones* that the hyphal cells in the host tissue are binucleate. He says, "whether this is true of all the cells it is impossible to say, but certainly a majority of the hyphal cells show two nuclei closely associated."

The binucleate cell condition in *Urocystis Cepulae* does not seem to arise at any one point in the mycelium. I am confident that cell fusions do not occur, as Rawitscher (*39*) described for *Ustilago Maydis*, at the time of spore formation. In all the numerous preparations of the parasitic mycelia that were examined, not one case of cell fusion between uninucleate mycelial cells was observed. Clamp connections such as were reported by Osner (*33, p. 212*) for *Ustilago striaeformis* are not present in *Urocystis Cepulae*.

The nuclear phenomena in *U. Cepulae* agree in the main with those of *Doassansia Sagittariae* as described by Rawitscher (*41*) in 1922, when he states that the binucleate condition arises a little before spore formation.

## SPORE AND SPORE-BALL FORMATION

Kühn in 1858 was the first to describe spore formation in

the genus *Urocystis*. Spore formation has been described for the following species: *Urocystis occulta*, Kühn (*27*) 1858; Wolfe (*51*) 1873; *U. Colchici*, Winter (*50*) 1876; *U. Violae*, Prillieux (*38*) 1880, Dangeard (*11*) 1894, Paravicini (*34*) 1917, Rawitscher (*41*) 1922; *U. Anemones*, Lutman (*29*) 1910, Paravicini (*34*) 1917; *U. Cepulae*, Thaxter (*47*) 1889, Whitehead (*49*) 1921, Anderson (*1*) 1921.

Prillieux (*38*) states that the sporogenous filaments form the fertile spores acrogenously, and agrees with de Bary (*16, p. 125*) 1866, that the sterile cells are formed by short hyphae which fix themselves to the surface of the young spore. Dangeard (*11*) in 1892 observed that the young spore cell contains two nuclei which fuse, and states that the sterile cells contain no nuclei. He thinks that "elles servent de nourriture aux cellules fertiles" (*p. 261*).

In *Urocystis Anemones*, Lutman (*29*) states that the fertile spores, together with the pseudo-spores, develop directly from binucleate cells. "Those that will become sterile contain little stainable material while the cytoplasm of the fertile spore is quite dense," (*p. 1214*). Lutman agrees with Dangeard that the two nuclei in the fertile cell fuse into one. Paravicini (*34*) and Rawitscher (*41*) in general confirm Dangeard's and Lutman's observations.

*Spore formation in Urocystis Cepulae.* In *U. Cepulae* the hyphae of the strands of mycelium crowded between the cells of the host plant in the sorus primordium finally begin to curve and coil in a characteristic manner (FIGS. 6, 7, PLATE 4). This feature, and also the increased tendency of the cytoplasm to take the stain, are characteristics which serve to distinguish the first differentiation of the young sorus. A study of these sporogenous hyphae shows abundant cases of the early stages of the developing spore balls.

Success in staining these stages requires rather thin sections (three microns thick), and the proper concentration of the gentian violet and orange G stains. These concentrations were so manipulated that the nuclei and cell septa were distinguishable at all stages.

The spore ball has its beginning with the production of short hyphal branches which may be designated as sporogenous branches. These sporogenous branches form a sort of corymbose

branching system, each branch of which consists of short cells that are binucleate. The spore ball primordia are differentiated from the surrounding hyphae in the sorus by their more densely stained cell cytoplasm, and a decided curving of the hyphal branches, even before the central fertile cell of the individual spore ball is distinguishable. In a single young sorus great numbers of spore balls can be observed in all stages of development.

The definitive fertile cell differentiates itself from the cells of the same branch and the cells of other adjacent sporogenous hyphae by a notable increase in size. In FIG. 6, PLATE 4, the central cell is shown slightly larger than the cells of the surrounding hyphae. All the cells are plainly binucleate. In sections of this thinness all the branches of a single spore ball are not included. The spore ball primordium measures about 10 microns across, the hyphal cells about 1.5–2 microns. In FIGS. 7 and 8, PLATE 4, the central cell has increased perceptibly in size.

At this stage of development careful study was given to determine whether cell fusions of any sort occur. Since the sporogenous branches curve back on themselves, one may get the impression that some form of cell fusion may be taking place, but such is not the case. The young fertile spore cell takes its origin from a binucleate centrally located cell of a sporogenous branch. The rapid development of the fertile cell results in crowding the adjacent cells as indicated in FIG. 9, PLATE 4, where the two ends of the original sporogenous branch are still recognizable and attached to the young spore cell.

The pseudo-spore primordia, as well as the central fertile cell, are at first binucleate (FIGS. 6–10, PLATE 4). This observation agrees with those of Lutman (*29*) and Paravicini (*34*) for *U. Anemones* and Rawitscher (*41*) for *U. Violae*. In FIG. 10, PLATE 4, the only apparent difference in the cells of the sporogenous branches is in the shape and size of the fertile spore cell as compared with the pseudo-spore primordia, the cells of which have not increased in size perceptibly. From this time on, the fast growing central cell tends to suppress the surrounding cells of the sporogenous hyphal branches. The contents of the surrounding cells, as Dangeard (*11*) holds, apparently serve as food supply for the developing fertile cell. It seems

probable that all of these binucleate cells of the young spore ball are potential spores. One of them begins to grow at the expense of the others, because it happens, very likely, to be in a more favorable position for food supply. As the central cell enlarges, the surrounding hyphal cells—the primordia of the pseudo-spores—become appressed to its surface. In FIG. 10, PLATE 4, the central cell is still binucleate and shows its attachment to the original sporogenous branch, while the primordial cells of the pseudo-spores have not increased perceptibly in size.

FIGS. 8–16, PLATE 4 are of the same magnifications, with the exception of FIG. 10, and serve to give an accurate representation of the rate of growth of the central fertile cell as compared with the growth of the pseudo-spores.

When the fertile spore cell has increased in size to about four microns in diameter, the two nuclei meet near the center of the cell and fuse (FIG. 11, PLATE 4). The stage at which fusion occurs agrees with that described by Dangeard (*11*) and by Rawitscher (*41*) for *U. Violae* and that described by Lutman (*29*) and by Paracivini (*34*) for *U. Anemones*. Because of the minuteness of the nuclei, details of the nuclear fusion could not be worked out.

With the stage of the nuclear fusion in the central cell, the pseudo-spores have increased in size and their two nuclei, in some cases, are pressed together (FIG. 12) giving the pseudo-spores the appearance of uninucleate cells. In FIG. 13, PLATE 4, the young spore measures 9.5 microns in diameter, the fusion nucleus one micron, while the whole spore ball is 12 microns across. In FIG. 14, PLATE 4, the fertile cell is surrounded by a darkly stained substance which is the first indication of the developing spore wall. The spore-wall substance is evidently formed from the fertile spore and is at first in a gelatinous condition which perhaps causes the surrounding pseudo-spores to become fastened to its surface. The spore wall increases rapidly in thickness (FIGS. 15, 16, PLATE 4) and hardens into a dense opaque substance to which the sterile cells are firmly attached.

Soon after the fusion of the two nuclei in the central cell, the protoplasts of the young pseudo-spores stain as if they are degenerating. Their nuclei become smaller and smaller (FIG. 13) and finally disappear (FIG. 14, PLATE 4). Their cytoplasm

becomes less and less dense and also finally disappears like the nuclei, leaving only the cell walls.

A mature spore ball of *Urocystis Cepulae* is composed of a fertile spore cell which is surrounded by a varying number of pseudo-spores. Spore balls taken from a mature pustule and mounted in distilled water measure from 19 to 20 microns in diameter, while the central fertile spores measure from 12 to 15 microns. Anderson (*2*) measuring fifty spores of *U. Cepulae* in lactophenol found the average diameter to be 16.15 microns. In fact, I find that the size of the spores of *U. Cepulae* approaches more nearly the size of the spores of *U. magica*, which Anderson gives as 22.19 microns.

In all the sections of developing and mature spore balls of *U. Cepulae* which I have examined, I have observed but one fertile spore cell in each spore ball. Great masses of these spore balls are formed in the mature pustule and are set free by the drying up and subsequent cracking of the host tissue.

## DISCUSSION

My studies of *Urocystis Cepulae* show conclusively that the two nuclei that fuse in the young smut spore are not derived from a fusion of cells of the saprophytic mycelium, nor from a conjugation of conidia, since such processes do not occur in this species. The binucleate cells originate in the parasitic mycelium in the stages preceding the differentiation of the sorus primordium. Just how the binucleate condition arises has not been determined. The haploid stage extends over a considerable portion of the life cycle of the organism including the whole of the saprophytic stage by which the fungus maintains itself in the soil probably for years.

The developmental history of *U. Cepulae* is quite different from that which has been described for certain apparently closely related species, viz., *U. Anemones* and *U. Violae*. According to Kniep (*26*) in 1921, the haploid stage of *U. Anemones* consists of but four cells. The diploid stage begins with the conjugation of the three or four promycelial branches. Rawitscher (*41*) in 1922 gives a similar description for *U. Violae*, except that in this species seven or eight promycelial branches (conidia) are produced.

In its haploid stage *Urocystis Cepulae* more nearly resembles

*Doassansia Sagittariae*, as described by Rawitscher (*41*) in 1922, but in *D. Sagittariae* uninucleate conidia are produced, though, as is the case in *Ustilago Maydis*, they do not conjugate. Rawitscher further states that the binucleate condition originates a little before spore formation. It is his opinion that the binucleate condition is brought about by a dissolution of the cross-wall between two cells (*p. 292*), but he does not support this contention with figures. Rawitscher allies *D. Sagittariae* with *Ustilago Maydis* in its developmental history.

In my opinion, *Urocystis Cepulae* cannot be allied with *Ustilago Maydis* in its life cycle, since in the former the binucleate condition arises in the vegetative hyphae soon after infection, and not at time of spore formation, as described for *U. Maydis*. In this connection it is interesting to note that the phenomenon of the gelatinization of the cell septum (or the cell walls) of two adjacent uninucleate cells, by which the binucleate condition is initiated at time of spore formation, as maintained by Rawitscher (*39*) for *Ustilago Maydis*, has never been described for any other member of the Ustilagineae. Since this is a matter of such fundamental importance, verification of the observation for this or another species is desirable.

De Bary (*17*) states that in *Ustilago hypodytes* the membranes of the hyphae swell into a gelatinous substance so that each individual spore is soon surrounded by a broad hyaline gelatinous sheath. The definitive membrane of the spore is then formed on its outer surface inside the gelatinous envelope, which soon disappears as the spore matures (*p. 175*). De Bary's description of the gelatinization of the hyphal membrane is concerned with the separation of spore elements and the subsequent development of the spore wall, and not with cell fusion as maintained by Rawitscher for *Ustilago Maydis*.

In the light of the remarkable results reported by Sartoris (*42*) in 1924, one is inclined to wonder whether the binucleate structures which originate as the result of conjugation of conidia obtained on artificial media are viable and able to infect the host. Sartoris has shown convincingly that the secondary spores formed from the fused conidia of *Tilletia Tritici* are unable to infect the host. By growing *Ustilago Zeae* in a solution of maltose to which potassium dihydrogen phosphate and magnesium sulphate had been added, Sartoris obtained conjugation of

the conidia. Mycelium grown on an alkaline glycerin agar with special subsequent treatment formed smut spores. But "the spores are physiologically unlike the chlamydospores formed on the host plants . . . they are like them in appearance but these chlamydospores of *Ustilago Zeae* do not form a typical promycelium" (*p. 636*).

Since the conidia of *Ustilago Maydis* will not conjugate in ordinary media (dung-decoction)according to Brefeld (*9*) Rawitscher (*39*) and others, the conjugation of the conidia on 'special media' is significant. Obviously Sartoris (*42*) believes that his results indicate that the conjugation of the conidia of *U. Maydis* is induced when a sufficient amount of nutrient material is not available for the fungus. This coincides with statements of Brefeld (*9*) Dangeard (*11*) Harper (*23*) and Lutman (*29*). Sartoris also agrees with Lutman that the conjugation of the conidia represents an old form of sexuality which has ceased to play an essential rôle in the life cycle of the smuts (*42, p. 638*). According to Bauch (*5*) in 1923, in *Ustilago longissima* the binucleate cells conjugate freely with each other—a condition one would not expect of binucleate cells.

Thus the question arises: are the binucleate structures observed by Kniep (*26*) in *Urocystis Anemones* and by Rawitscher (*41*) in *U. Violae* viable and able to infect the host, or are they merely responses of the fungus to abnormal conditions? On this point the papers of Kniep and Rawitscher do not make any definite contribution. Without making any infection experiments, they assume that the binucleate structures (binucleate conidia) which originate by the conjugation of the mycelial branches of these species, will infect the host and thereby initiate the binucleate condition in the parasitic mycelium. Kniep and Rawitscher evidently based their conclusions as to the origin of the two nuclei that fuse in the young spore in *U. Anemones* and *U. Violae* on the following data: In *Ustilago Tragopogonis*, Rawitscher (*39*) in 1912, observed that the mycelium infecting young buds of *Tragopogon pratensis* is intercellular and is composed of binucleate cells. The binucleate condition of the mycelial cells and thus the young spore cells was considered by him to be brought about by the conjugation of conidia. Since, however, he did not include infection experiments in his work, one cannot be sure that this is the case. In 1922 Rawitscher

(*41*) extended his observations to *Tilletia Tritici* and *Cintractia Montagnei* with similar interpretations.

Rawitscher's contention that the conjugation of the conidia initiates the binucleate condition received substantial support by the further work of Kniep. In 1919 Kniep (*25*) produced evidence of a physiological nature in support of the belief in the sexual nature of the conidia that conjugate, by presenting data indicating the differentiation of the so-called plus and minus strains in the anther smut. He reports that in the anther smut two kinds of conidia arise from the promycelium. They are externally alike but physiologically different. Conjugation takes place only when the two different kinds of conidia are brought together. Conidia and cultures arising from a single conidium do not conjugate. Therefore, there exists between certain conidia a difference which he terms 'physiological sex differentiation.' In support of these statements Kniep isolated a great number of 'single conidial cultures' of the anther smut from spores obtained from various host plants, and succeeded in bringing about conjugation in the majority of cultures by combining the conidia with suitable mates. These observations of Kniep, together with his conclusion that *Ustilago violacea* is composed of biological forms, have been confirmed and extended by Bauch (*4*, *5*, *6*) and by Zillig (*53*). The work of these investigators seems to establish the sexual nature of conidial fusions, but it is evident that the nuclear history of a larger number of species must be worked out before full agreement as to the nuclear phenomena in the reproduction of the Ustilagineae can be expected.

The results of my study of *Urocystis Cepulae* conclusively show that the uninucleate saprophytic mycelial cells taken from cultures infect the host tissue and produce uninucleate parasitic mycelium in the host tissue, which, in turn, soon gives rise to binucleate mycelial cells. As stated above, all the cells of the young sorus of *Urocystis Cepulae* are binucleate, and these binucleate cells give origin to the spore ball primordia—the fertile spore itself taking its origin from one of the binucleate cells.

## SUMMARY

1. Onion seedlings are infected by *Urocystis Cepulae* as they make their way through the soil. Infection occurs only through

the cotyledons. Within three to four weeks mature pustules appear on the cotyledons of the seedlings.

2. Mature spores from unopened sori were germinated in onion decoction or on onion agar. In the course of three to seven days the spores begin to germinate. All the spores do not germinate at once.

3. Germination begins by the protrusion of a hemispherical promycelium which buds out eight or a less number of hyphae of indeterminate growth. By continued growth and branching of these primary hyphae, a weft of mycelium is produced about the spore which is visible to the eye in about ten days. Conidia are not produced.

4. The smut fungus was isolated and grown on various media as follows: onion agar, sterile potato, onion, bean, and carrot.

5. During spore germination the fusion nucleus divides within the spore. The nuclei migrate through the short promycelium to the promycelial branches. One nucleus passes into each promycelial branch, which segments ultimately into uninucleate cells. Conjugation between promycelial branches does not occur.

6. The saprophytic mycelium is uninucleate from the beginning and remains so throughout its existence.

7. Each mycelial cell of the saprophytic mycelium may function as an oidial spore. The oidial spores serve to propagate and disseminate the fungus in the soil.

8. The oidial spores are uninucleate and after germinating produce uninucleate mycelium, as do the resting spores.

9. Dessication does not kill the saprophytic mycelium nor the fungus in the soil.

10. Infection of the seedlings takes place directly from the mycelium, under experimental conditions, and probably in the field likewise.

11. The parasitic mycelium is intercellular throughout its existence.

12. The hyphal cells of the parasitic mycelium are at first uninucleate but become progressively binucleate as they approach the young sorus primordium, where all the cells of the sporogenous hyphae are binucleate.

13. The spore ball has its origin from a group of sporogenous branches, each of which consists of short binucleate cells.

14. The fertile spore takes its origin from a binucleate, centrally located cell of a sporogenous branch.

15. The two nuclei in the young spore soon fuse. The spore cell then increases rapidly in size, the pseudo-spores become appressed upon its surface.

16. The pseudo-spores have their origin from binucleate cells of the sporogenous branches, and serve as 'nurse' cells to the central fertile spore.

This work was done under the direction of Professor R. A. Harper, to whom I wish to express my sincere thanks and appreciation for his unfailing interest, valuable advice, and stimulating criticism during the progress of this problem.

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## Description of plates 1–4

The photographs were made with Carl Zeiss Protar and Planar lenses, having 6½ inches and 4 inches focal length respectively. Photographs were made natural size except where otherwise indicated: all have been slightly reduced in reproduction. All figures were drawn with the aid of the camera lucida and Zeiss 2 mm. apochromatic objective N. A. 1.30 and no. 12 compensating occular.

### Plate 1

FIGS. 1–6. Mycelium of *Urocystis Cepulae* growing on onion agar, representing various ages of the culture.

FIG. 1. Culture transferred from onion agar plate to onion agar, 11 days after germination. Photographed 2 days after transfer. A spherical mat of mycelium, white in appearance.

FIG. 2 Culture growing 3½ days after transfer. The mycelial threads can be observed to be growing radially from the oval cottony mass of mycelium. × 5.5.

FIG. 3. The largest culture is 19 days old and developed from a single spore. The three upper and smaller mycelial mats represent secondary infection and are about 8 days old. Ridges on the surface of each.

FIG. 4. This represents a culture 16 days old, on onion agar. The characteristic wrinkles on the surface are apparent. A delicate fringe surrounds the periphery. × 2.

FIG. 5. Culture transferred from culture on bean to onion agar. Fourth transfer from the original culture. Culture 30 days old. × 2.

FIG. 6. Culture growing on onion agar, 69 days old. The characteristic ridges running radially. Along the lower edge is a fringe of growing hyphae that show zonal growth.

FIG. 7. *U. Cepulae* growing on sterile potato, 7 days' growth. The mycelium is snow-white and tends to accumulate in nodular masses. × 5.

FIG. 8. Culture on potato, 51 days old. Because of certain conditions in the potato, the mycelial growth is being slowly destroyed. The mycelial mat at this stage is thin and the crests of the reduced ridges have broken open.

FIG. 9. Culture on sterilized onion, 68 days old. Growth at first was exceedingly slow. Finally a mat of mycelium appears with the characteristic ridges and wrinkles.

FIG. 10. Culture on a green but sterile onion, 47 days old. The mycelial mat covers a portion of the onion. Surface shows wrinkles, the crests of which are white. Spore formation does not occur. Second transfer.

## Plate 2

FIG. 1. *Urocystis cepulae* growing on sterilized bean pod, 7 days old. The mycelium piles up in layers to a depth much greater than on any other medium used. The whole mass appears white, due to the loose superficial hyphae which enclose air in the meshes of the mycelium.

FIG. 2. Culture on sterilized bean pod, 51 days old. The ridges on the surface of the mycelial mat are high and thick. The growth is rank. At the periphery of the culture is a fringe of actively growing mycelium which shows zonal growth.

FIG. 3. Culture on sterilized bean pod, 51 days old. Mycelium which was growing on onion agar was broken up into its cellular elements then sprayed on to the bean pod. Apparently wherever a mycelial cell (oidial spore) settled, it developed a weft of mycelium. Great numbers of the little mycelial colonies have fused.

FIG. 4. Culture on sterilized carrot, 25 days old. Due to slow growth on carrot, in the beginning, the mycelial mat is just commencing to show the characteristic wrinkled surface.

FIG. 5. Culture on sterilized carrot; later stage than FIG. 4, 47 days old. The characteristic wrinkling is evident. The wrinkles are not nearly so pronounced as on the onion agar or on the bean.

FIG. 6. Culture on sterilized carrot, 61 days old. The ridges have fused to such an extent that the whole surface is much smoother—just the opposite from that noted on the bean, FIG. 2.

FIG. 7. Mycelium growing on onion agar. Each mycelial mat developed from a mycelial cell (oidial spore). The surface appears wrinkled.

FIG. 8. Mycelium developed from spores taken from an unopened smut pustule, then placed on onion agar. Thus the mycelium in FIG. 7 is from oidial spores while that of FIG. 8 is from the resting spores.

## Plate 3

FIG. 1. Germinating spore of *U. Cepulae*. The subspherical promycelial vesicle is protruding from one side. The promycelium is budding from its surface the first primary hypha. × 960.

FIG. 2. A later stage. Two primary hyphae are growing from the surface of the promycelium. × 960.

FIG. 3. A spore with the promycelium. From the promycelium has developed one primary hypha, which consists of two cells, each uninucleate. The cell nearest the promycelium is budding off a branch.

FIG. 4. A later stage of germination than FIG. 3. Two primary hyphae are shown; one is branched and consists of uninucleate cells. × 960.

FIG. 5. The germinating spore with hemispherical promycelium. Four primary hyphae are growing from the promycelium. The primary hyphae consist of uninucleate cells. × 960.

FIGS. 6, 7, and 10. Cells of the saprophytic mycelium in different stages of activity. × 1200. In older cultures some of the hyphae are composed of uninucleate cells of the appearance of FIG. 7, cytoplasm is dense and contains refractive granules. Other hyphae may consist of cells rounded at the ends and constricted in the middle, FIG. 6. Still others more desiccated, FIG. 10, which appear like a chain of oidial spores. Each cell is uninucleate.

FIG. 8. Hyphal cell germinating. The mycelial cells had been shaken apart and placed in onion decoction for 18 hours. The nuclei and cytoplasm have left the original cell and, in each case, migrated into the branch. × 900.

FIG. 9. Represents the characteristic habit of the saprophytic hyphae, showing also the characteristic branching. Each cell of the hyphae is uninucleate. The hypha, as represented, is from the aerial portion of the culture, growing on onion agar. × 1200.

FIG. 11. A germinating hyphal cell (oidial spore) in which the mother cell and the branch each contain a nucleus. A septum has not as yet been laid down between the nuclei. × 1200.

FIG. 12. A portion of a mycelial thread which shows each uninucleate cell of the hyphae germinating in a characteristic fashion. × 900.

FIG. 13. Mycelial cells recovered from the soil 10 hours after the soil had been infected with mycelium from the cultures. Each cell is beginning to germinate. × 1200.

FIG. 14. Similar to FIG. 8 except that the nuclei have remained in each of the original mycelial cells (oidial spores). One cell is shown which is giving off a germ tube at each end. × 1200.

**Plate 4**

FIGS. 1–3. Binucleate cells of the parasitic mycelium that permeate the host tissue in the immediate vicinity of the young sorus. × 1200.

FIGS. 4, 5. Uninucleate cells of the vegetative mycelium. × 1200.

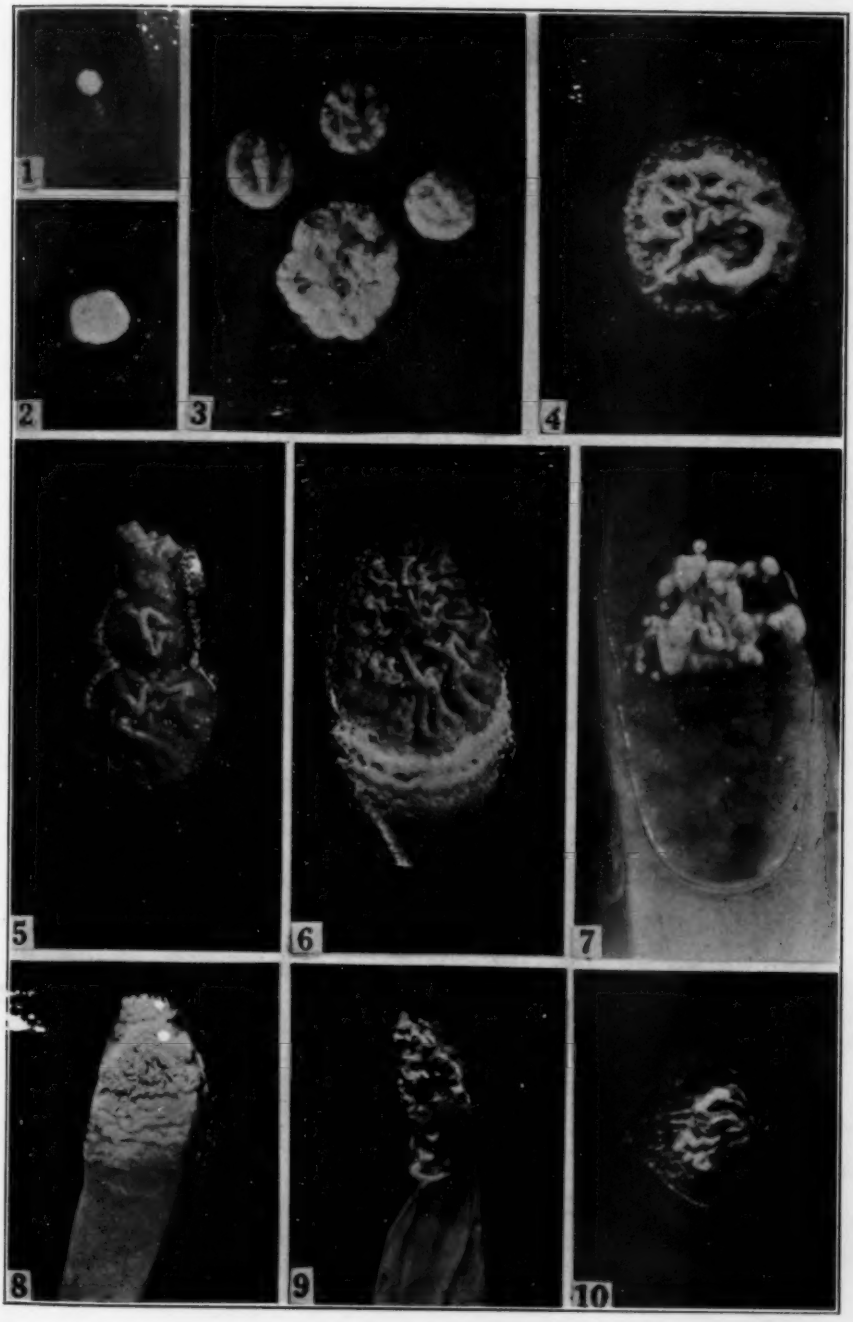
FIG. 6. Spore ball primordium measuring about 10 microns across; the hyphal cells one-half micron in diameter. The hyphal cells are binucleate. The large central cell is the primordium of the fertile spore. × 1200.

FIGS. 7–10. Spore ball primordium showing somewhat later stage. The binucleate central cell has increased in size. The cells of the surrounding sporogenous hyphae are each binucleate. FIG. 9. shows the young binucleate central cell increasing in size, causing the sporogenous branch from which it originated to be distorted. The two ends of the sporogenous branches are still attached to the young spore. The central cell measures 2 × 3 microns. (FIGS. 7–9, × 1200, FIG. 10, × 900.)

FIG. 11. The two nuclei are in close proximity and apparently fusing. The surrounding hyphal cells are binucleate.

FIGS. 12, 13. The now uninucleate central cell has increased in size while the pseudo-spores appear uninucleate with degenerating cellular contents. × 1200.

FIGS. 14–16. The central spore has increased to such proportions that it presses against the pseudo-spores which now adhere firmly to its surface. The psuedo-spores now contain no nuclei and very little cytoplasm. × 1200.

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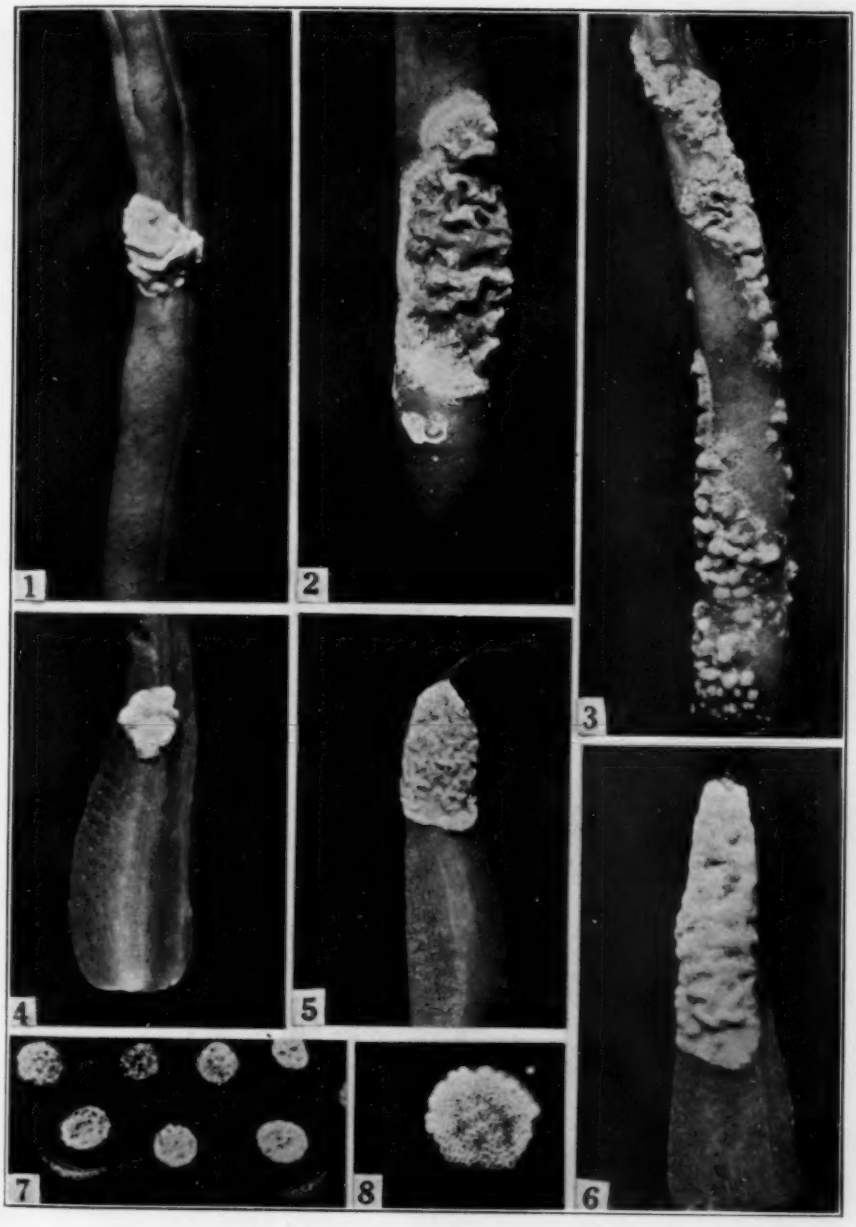
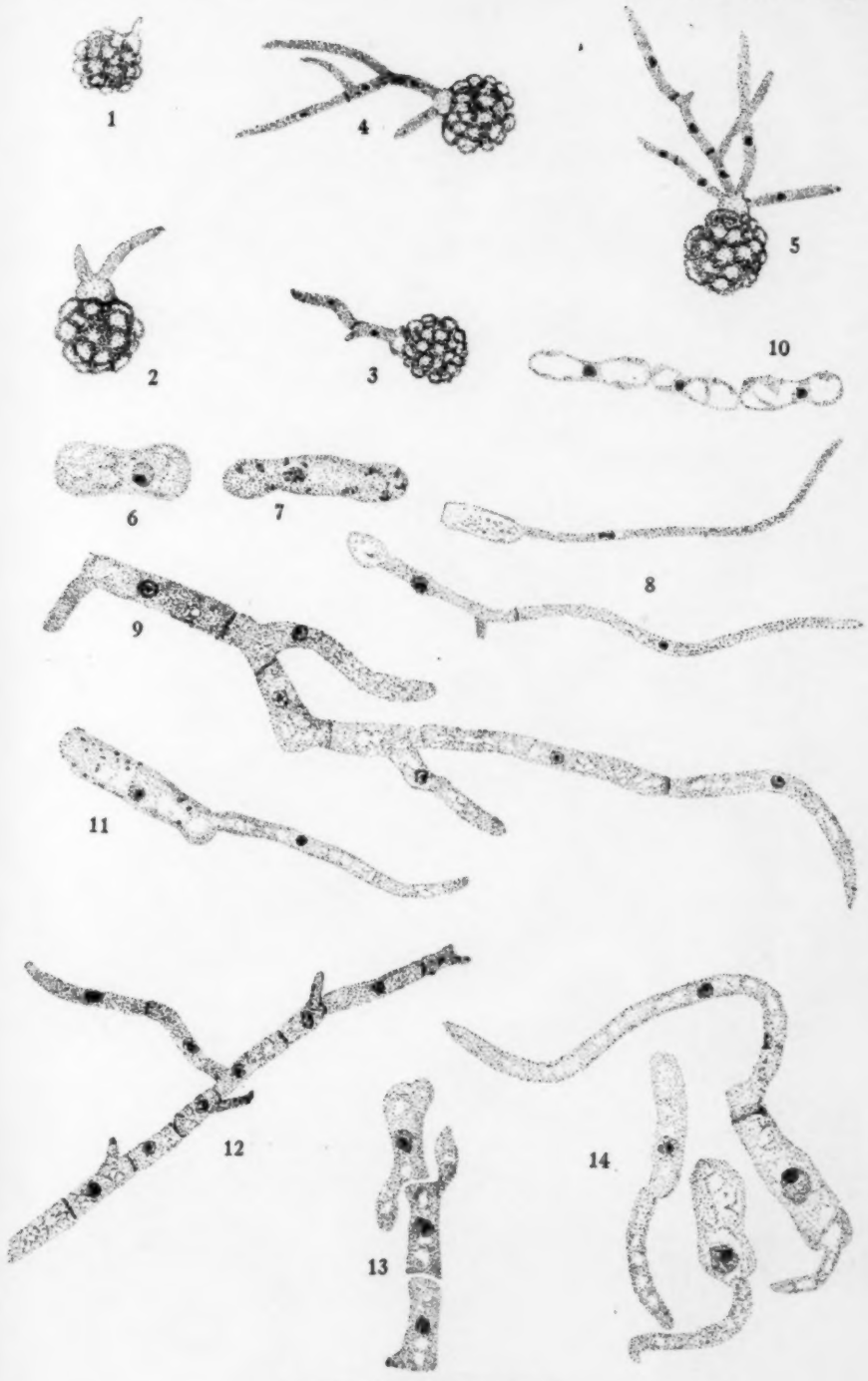



CULTURES OF UROCYSTIS CEPULAE




UROCYSTIS CEPULAE Frost

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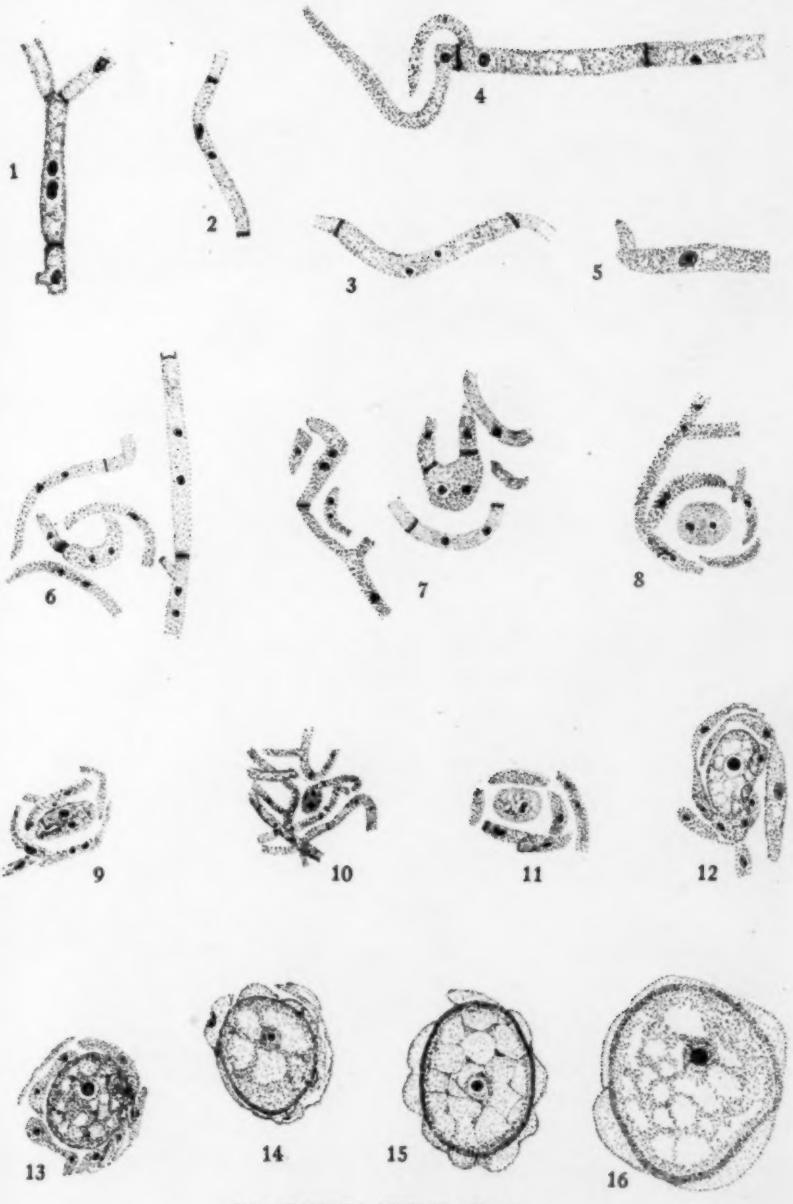


UROCYSTIS CEPULAE FROST

# INDEX TO AMERICAN BOTANICAL LITERATURE
## 1925

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